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Work Assignment #9

ADULTERATED FISH WASTES OCEAN DUMPING OPERATIONS

Prepared For:

OCEAN AND ESTUARIES SECTION
U.S. ENVIRONMENTAL PROTECTION AGENCY REGION IX

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Under Subcontract To:

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September 22, 1989

Mr. Raymond E. Baum, Jr.
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U.S. Environmental Protection Agency
499 South Capital Street, S.W.
Washington, D.C. 20460

Subject: Final Report on Adulterated Fish Wastes Ocean Dumping Operations under contract number 68-03-4045, Work Assignment #9

Dear Mr. Baum:

Enclosed is a copy of the "Adulterated Fish Wastes Ocean Dumping Operations - Final Report" which was prepared by National Business Consultants/DC, Inc. under subcontract to Executive Resource Associates, Inc.

If you have any questions regarding the report, please call me at 920-5200.

Sincerely yours,

Charles A. Bakewell
Project Director

cc: Patrick Cotter

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SUMMARY

Wastes from the processing of fish for commercial use which have been pretreated through a Dissolved Air Flotation (DAF) process are in some cases disposed of through ocean dumping; a permit is required. Such wastes have a biochemical oxygen demand of approximately 150,000 mg/l, suspended solids of 130,000 mg/l, oil and grease of 14,000 to 18,000 mg/l, total nitrogen of 1,900 mg/l, and total phosphorus of 1,200 mg/l. The DAF sludge, a major component of wastes destined for ocean disposal, has an average bulk density of 0.89± 0.08 gm/ml to 1.00± 0.02 gm/ml; sea water has a density of 1.025 gm/ml. Thus, some of the waste tends to float on the ocean surface, at least until the air bubbles entrained within it are dispersed. Other solid particles within the waste sink.

Alum is used in the treatment process. The resulting concentration of aluminum in the waste is expected to be sufficient to be toxic to organisms within the confined quarters of a bioassay. Aluminum in the waste would not be expected to be toxic to organisms under conditions prevailing in the open ocean at a disposal site. The sludge, in a one-time 96-hour bioassay with mysid shrimp, had an LC_{50} of 400 mg/l. (LC_{50} is the concentration that is lethal to 50 percent of the test organisms in a specified time.)

The environmental effects of ocean disposal tend to be mitigated by a dispersive ocean environment. The Biochemical Oxygen Demand (BOD) creates a decrease in Dissolved Oxygen (DO) immediately after disposal, but field studies associated with dumping operations have not shown ambient DO concentrations below 5.5 mg/l as a result. Ammonia concentrations are elevated to maximum levels of 0.119 mg/l and 0.41 mg/l for short time periods

following dumping, but the ammonia is reduced to ambient ocean levels within six to eight hours. No evidence of deleterious environmental stress has been observed during fish waste ocean disposal monitoring. There is a visible surface plume remaining for several hours following fish waste dumping, which shows as a light blue-turquoise patch on the ocean surface, and ammonia concentrations appear to be the best analytical tracer to track the floating plume movement.

Waste characterization prior to dumping should include periodic bioassays including certain chemical analyses of bioassay test waters; the waste should be analyzed for bulk density and certain traditional characteristics plus nutrients, oil and grease, aluminum, cadmium, and mercury. Dumpsite monitoring should include surface plume tracking through current drogues, transmissometer, or ammonia-N testing. The water column needs to be monitored only for temperature, pH, salinity, dissolved oxygen, ammonia-N, light transmissivity and visible depth, at least in deep (greater than 200 meters) open ocean water. Depending upon the particularities of individual dump sites, benthic organism sampling and assessment, benthic sediment characterization, chlorophyll a and marine phytoplankton identification, and sediment trap analyses may be desirable additions to the monitoring program.

Currently, no mathematical model exists to predict the settleable plume and ocean floor sediment deposition resulting from fish wastes. With substantial effort, an existing model might be adaptable to serve as a predictive tool. The greatest difficulty to overcome in such modification of an existing model would be the negative bulk density factors as related to sea water, and the

waste fractionation problem of associating time periods and fall velocity with the particles in a waste portion that temporarily float and those that sink during various time periods.

PURPOSES

The purposes of this report are to: (1) summarize the state of knowledge related to adulterated fish waste characteristics, the ocean dumping of such wastes, and potential oceanic environmental effects; and (2) provide monitoring recommendations for a generic dump site, which would include a discussion of any applicable model to track the plume and solids deposition from the dumping operation.

REGULATIONS

The Marine Protection, Research, and Sanctuaries Act of 1972, as amended, 33 U.S.C. 1401 et seq., provides that the Administrator of the U.S. Environmental Protection Agency may issue permits for the transportation from the United States of material for the purpose of dumping it into ocean waters. Regulations governing the dumping of such matter are to be found at 40 CFR 220.1 et seq.

The regulations exclude fish waste (40 CFR 220.1(c)):

> "This Subchapter H does not apply to, and no permit thereunder shall be required for, the transportation for the purpose of dumping or the dumping in ocean waters of fish wastes unless such dumping occurs in:
>
> (i) Harbors or other protected or enclosed coastal waters; or
>
> (ii) Any other location where the Administrator finds that such dumping may unreasonably be anticipated to endanger health, the environment or ecological systems."

Fish wastes are not further defined in law or regulation. Generally, fish wastes are taken to mean such wastes which have not been adulterated with additives. This report generally describes and addresses fish wastes to which have been added a coagulant and a polymer, and which result from operations within a processing plant such that they fit the definition of an industrial waste as provided by U.S.C. 1412a(d)(2).

WASTE SOURCES

Principal fish processing wastes included in a ocean dumping permit application generally are Dissolved Air Flotation (DAF) sludge, pre-cooker water, press water, and thaw water. DAF sludge is a sludge containing small fish solids, greases, oils, dissolved organic materials, and alum that are removed from wastewater in an air flotation treatment process. Pre-cooker water is the blood, scales, and juices that result from steam cooking of whole fish in a steam oven. Press water is the liquor squeezed from fish in a fish meal reduction press plant. Thaw water is the water resulting from thawing whole fish transported via vessel from a fishing ground; it contains scales, blood, flesh particles, and some juices.

The wastes described above are from the processing and canning of ocean fish in a fish processing facility. The highly odorous sludge from the DAF process must be disposed of; however, its land disposal, where disposal lands may be available, requires dewatering and the use of expensive odor masking chemicals. If the sludge is ocean dumped, the other fish processing wastes can be dumped with it for two principal reasons. Because of their high Biochemical Oxygen Demand (BOD) and the large quantities of solids that these wastes contain, they cannot be effectively treated by the Dissolved Air Flotation (DAF) process. Further, these wastes serve as a convenient means of

diluting the sludge so that it will discharge more effectively from the barge during the dumping process, and the sludge will mix more effectively with the sea water.

Harvesting of ocean fish involves netting, trapping, and line fishing. Fishing vessels use the latest technology for locating fish and harvest them in the most expedient and economical manner consistent with local regulations. Once aboard the vessel, fish are taken directly to the processor, or are iced or frozen for later delivery. Tuna, for example, are harvested by line or by net. They are frozen onboard the vessel and thawed, usually by salt water, at the processing plant (EPA, 1974).

Thawing may take place in large tanks and may consume two to six hours. Thawed fish are conveyed to butchering tables where tuna, for example, are eviscerated with the viscera dry-captured or screened from the waste stream and processed as a fisheries by-product.

Some fish, anchovy for example, are transported in the hold of a vessel. In the unloading operation, the holds are filled with local estuarine water and the resulting fish-water slurry is pumped over rotating or static screens to separate the fish from the bailwater. The fish may go to a fish meal reduction process facility. The resulting bailwater contains scales, slime, bits of fish flesh, and blood. (EPA, 1975).

Further processing of edible fish, such as tuna, includes some form of pre-cooking to prepare the fish for the picking and cleaning operation.

Pre-cooking facilitates the removal of skin, bone, gills, and other materials. Pre-cooking is done in steam cookers that have a capacity of 10 tons of fish per cook with the cook lasting 2 to 4 hours at a live steam temperature of 200°F. The steam condensate from the pre-cooking operation with fish oils and fluids is referred to as pre-cooker water or stick water.

The picking and cleaning tuna operation separates edible fish portions from non-edible portions. Heads, tails, fins, skin, and bones are manually removed. This scrap is collected at the leading end of long cleaning tables, and by means of an auger is conveyed to a collection area for transport to the fish meal reduction plant.

Edible tuna portions are placed in cans by automatic packing machines. The cans then are filled with soybean oil, a brine solution, and monosodium glutamate; the oil replaces the natural oils lost in pre-cooking and lubricates the tuna to prevent sticking to the sides of the cans during the high temperatures reached in retorting. After vacuum sealing in a lid seaming machine, the cans are run through a can washer to remove all of the particles and oil from the outside. Packed cans then go to large (4 1/2 ft. by 37 ft.) pressure cookers where the tuna are sterilized at 250°F for 90 minutes (EPA, 1974).

Following the cooking operation, the fish proceed to a battery of screw presses where liquid and solid portions of the cooked fish are physically separated into press cake and press liquor or water. The press cake is dried, ground into meal, and stored for shipment. Press water contains solid and dissolved fish protein, oils, fats, and ash. Oils and solids may be extracted

from the press liquor by use of centrifugal oil separators. The remaining press water, sometimes also called stick water, contains dissolved and suspended protein, fats, oil, and ash (EPA, 1975).

Fish wastewaters, then, originate from blood, scales, and juices from the thawing operation; blood, juices, and small particles from butchering; oils, meat, bones, and juices from pre-cooking; and soaps and detergents from can washing. Air flotation with appropriate chemical addition is a physical chemical treatment technology capable of removing high concentrations of solids, greases, oils, and dissolved organic material in the form of a floating sludge.

In the DAF process, wastewater with addition of alum and a polymer, is pressurized in the presence of air and then released to a flotation tank at ambient pressure. Small rising air bubbles dispersed throughout the flotation tank as a result of this process carry with them suspended material in the wastewater to form a floating sludge on the flotation tank. The floating, concentrated sludge then is skimmed off and this is the material that must be disposed of. As a result of this treatment, or pre-treatment, the BOD and suspended solids removals may attain 70 to 90 percent or higher in the wastewater. Such materials, of course, have been concentrated in the floating sludge (EPA, 1975).

WASTE CHARACTERISTICS

Fish processing wastes, including DAF sludge, have very high levels of BOD, total suspended solids, volatile solids, oil and grease, Chemical Oxygen Demand (COD), total organic carbon, organic nitrogen, ammonia nitrogen, and phosphorus.

BOD is the amount of Dissolved Oxygen (DO) used by bacteria to stabilize decomposable organic matter under aerobic conditions within the definition of the test, which generally is five days. BOD does not cause direct environmental harm, but it measures the amount of DO required within a receiving water to stabilize the organic material. The BOD of unpolluted water would be expected to be less than 2 mg/l. Generally, one pound of DO is required to stabilize one pound of BOD.

Total Suspended Solids (TSS) are the organic and inorganic suspended matter in water that potentially may settle to form a sludge blanket on the bottom of a water area, or while suspended will absorb light and create turbidity. Inorganic suspended solids include sand, silts, and clays; organic solids include grease, oils, and animal and vegetable fat.

Total Volatile Solids (TVS) represent the amount of organic matter within the solids fraction of a sample that is volatilized in 60 minutes at a temperature of 550^{o}C.

Oil and grease (O&G) is a self-explanatory term, which in the case of fish wastes refers to animal O&G. O&G exhibit an oxygen demand.

COD measures the total quantity of oxygen required to oxidize organic matter to carbon dioxide and water under severe chemical and physical conditions in the presence of a strong chemical oxidant. COD values are greater than BOD values and may be much greater when significant amounts of biologically resistant organic matter is present.

Total Organic Carbon (TOC) is a measure of the organic carbon content of a liquid. TOC represents a speedy and convenient way of estimating the degree of organic contamination.

Nitrogen (N) and phosphorus (P) are components of living matter. Beard (1926) reported that fish flesh is 2.5 percent (wet weight) nitrogen and 0.2 percent phosphorus. Borgstrom (1961) reported total nitrogen in fish as ranging from 2.83 percent for Atlantic cod to 3.46 percent for sardines. McGauhey et al. (1963) reported that the nitrogen phosphorus content in trout is about 3 percent N and 0.2 percent P by weight. As a point of reference, 1 percent is equivalent to 10,000 parts per million. Nitrogen in seawater is found at 0.03 to 0.9 mg/l and phosphorus is found at 0.001 to 0.10 mg/l (Todd, 1970). Total Kjeldahl Nitrogen (TKN) measures organic plus ammonia nitrogen. Ammonia nitrogen is a common product of the decomposition of organic matter. In the presence of DO, ammonia (NH_3) is converted to nitrite (NO_2), then to nitrate (NO_3) by nitrifying bacteria. Nitrification of organic nitrogen and ammonia by indigenous microorganisms creates a demand on DO resources. Both N and P are required for life. Elevated levels of N and P result in water enrichment and may produce excessive microorganism growths or "blooms".

DISSOLVED AIR FLOTATION SLUDGE

In quarterly analyses of DAF sludge (eight samples in total) submitted to EPA Region IX during 1985 and 1986, Star-Kist Foods, Inc., American Samoa facility, reported the following values in mg/l:

	Maximum	Minimum	Mean
BOD	268,958	73,167	150,000
TSS	132,373	76,700	95,950
O&G	29,578	8,209	18,180
N	6,822	508	1,890
P	1,435	1,113	1,263

When data from this facility are expanded to include the last quarter of 1980 through the first quarter of 1987, a total of 26 sampling periods, the following values have been reported in mg/l:

	Maximum	Minimum	Mean
BOD	268,058.3	102,500.	160,715.2 $\pm$ 36,487.3
TSS	161,215.	18,030.	105,858.7 $\pm$ 33,365.3
O&G	39,722.5	8,209.2	18,824.7 $\pm$ 7,226.3
N	6,822.7	578.2	1,794.9 $\pm$ 1,291.1
P	2,214.7	646.	1,091.5 $\pm$ 353.4

Of interest, also, is the sludge density. Here, 42 analyses of various American Samoa fish cannery wastes sludges reported from 1981 through 1986 indicated a mean density of 0.935 gm/ml. The maximum density reported was 1.06 gm/ml; the minimum was 0.72 gm/ml. One facility had a bulk density of 0.89 $\pm$ 0.08 gm/ml; the other, 1.00 $\pm$ 0.02 gm/l.

Soule and Oguri (1982) report on three samples collected in July, 1979, from the Van Camp Samoa facility and three samples from the Star-Kist Samoa facility with the following results:

	Maximum	Minimum	Van Camp Mean	Star-Kist Mean
BOD, mg/l	258,000.	105,000.	225,000.	142,000.
TSS, w/w%	21.4	9.6	18.5	14.1
Vol Solids, % of Suspended solids	96.5	79.4	95.5	86.5
TKN, mg/l*	769.	587.	678.	621.
P, mg/l*	1,031.	739.	804.	793.

	Maximum	Minimum	Van Camp Mean	Star-Kist Mean
Bulk Density, gm/ml	1.02	0.77	0.893	0.958
pH, Units	6.2	5.8	6.1	5.9
Aluminum, mg/kg	10,400.	711.	5,770.	1,260.
Cadmium, mg/kg	6.4	1.3	3.5	3.3

* The reference listed N and P in mg/kg, which normally is a dry weight designation. It has been assumed that the analyses represent mg/l. For example, if the dry weight solids were 10 percent, 1 mg/l would convert to 10 mg/kg dry weight; if the dry weight solids were 4 percent, 1 mg/l would correspond to 25 mg/kg dry weight. The dry weight solids value was not published. To consider the value's dry weight would not harmonize with the quarterly analytical data reported earlier in this section.

Soule and Oguri (1982) also published analyses from four samples of DAF sludge from Terminal Island, CA, with the following constituent values:

BOD, mg/l	84,000 to 761,000
Total solids	22 to 25 percent wet weight
Vol. solids	83 to 87 percent of suspended solids
Total P, mg/l	480 to 1,290
Aluminum, mg/l	29 to 514
Cadmium, mg/l	0.09 to 0.8
Bulk density, gm/ml	0.764 to 0.830

In 1983, Soule and Oguri presented results of 10 analyses on waste material from Star-Kist, Somoa, that was disposed of at sea during October, 1980, to March, 1983. The analyses represented quarterly sampling of the waste material; values are in mg/l unless otherwise noted:

	Maximum	Minimum	Van Camp Mean
BOD	188,000.	137,000.	154,000.
TSS	219,000.	78,000.	131,000.
O&G	20,100.	6,500.	14,300.
TKN	2,554.	574.	1,266.
P	1,785.	661.	945.
Bulk density, gm/ml	0.96	0.72	0.83

Thus, data for DAF sludge indicate a decomposable organic material with a high BOD of 150,000 mg/l, and high solids, O&G, nitrogen and phosphorus. The bulk

density appears to range from 0.72 to 1.06 and average 0.83 to 0.94 gm/ml. Aluminum appears to be the only non-biodegradable substance of concern; it has been added to the sludge in the form of alum.

Aluminum sulfate hydrate at a dosage of 200 to 300 mg/l is added to the fish processing wastewater as a coagulant prior to pressurization in the presence of air and released in the flotation tank; the compound contains 17 percent Al_2O_3 (Wass, 1983). At a concentration of 300 mg/l, there would be 51 mg/l Al_2O_3, or 27 mg/l Al in the sludge. The aluminum concentrations reported by Soule and Oguri (1982) were substantially higher than this calculated value; they ranged from 29 to 514 mg/l.

In a one-time 96-hour acute static bioassay, Star-Kist, Samoa, DAF sludge has an LC_{50} (the concentration that is lethal to 50% of the test organisms in a bioassay within the time specified for the test and under the conditions of the test) of 0.040 percent sludge to the mysid shrimp, 0.46 percent to a planktonic copepod, and 0.46 percent to the California killifish (Soule and Oguri, 1983). Thus, the sludge LC_{50} is 400 mg/l for the most sensitive species. The specific cause of the test organism mortality was not identified; aluminum would be suspect.

OTHER WASTES (FROM SOULE AND OGURI, 1982)

Analyses of four to six samples of pre-cooker juice, the broth that results from the steam cooking of whole raw tuna in steam ovens, indicated;

Fat	1% by volume
Solids	6% by volume

Water	93% by volume
TSS	3,020 to 17,220 mg/l
BOD	2,600 to 39,650 mg/l

Similar sample analyses of press liquor, the material squeezed from the press in a fish meal reduction plant, showed:

Fat	12% by volume
Solids	12% by volume
Water	76% by volume
TSS	4,860 to 18,060 mg/l
BOD	26,300 to 69,800 mg/l

Tuna brine thaw water analyses of 15 to 17 samples indicated:

BOD	3,400 to 57,000 mg/l
TSS	333 to 5,000 mg/l

Mackerel unloading water, seawater pumped into the hold of mackerel fishing boats so that the catch can be off-loaded by vacuum pump, in 10 samples analyzed showed:

BOD	827 to 3,400 mg/l
TSS	333 to 2,540 mg/l

Anchovy unloading water, seawater pumped into the hold of anchovy fishing boats so that the catch can be off-loaded by vacuum pump, in three samples analyzed indicated:

BOD	34,000 to 46,000 mg/l
TSS	4,344 to 18,000 mg/l

DUMPING PROCEDURE

Typically, the dumping barge circles inside a 1.5 nautical mile designated dump site while discharging wastes through discharge ports in the hull bottom, which has an 8.5 foot draft. Initial mixing is by turbulence from the hull, the vessel propellers, and by discharge velocity. The dumping of a barge load takes from 30 to 60 minutes during which time 15,000 to 30,000 gallons of waste may be discharged.

ENVIRONMENTAL EFFECTS

In accordance with the provisions of Section 74 of the Clean Water Act of 1977, EPA submitted a report (1980) to Congress on the ecological consequences of marine disposal of seafood processing wastes. Section 74 specified that EPA should focus its study on untreated seafood waste discharges. The report concluded that some coastal areas can assimilate or disperse large amounts of waste without serious effect, while other areas are adversely impacted. The two most significant site-specific factors identified by EPA were the amount of waste discharged and the hydrological conditions of the receiving waters, i.e., the concepts involving loading and assimilative capacity.

The types of harmful effects specified during the EPA study included:

1. Solids accumulation, which leads to smothering of bottom dwelling organisms with possible negative effects on the quality of the water above as well.

2. Excessive oxygen demand, which is the result of bacterial decomposition of the waste.

3. Excessive oil discharge, which may produce floating oils that damage marine birds, shoreline property, and boats.

4. Aesthetic effects, which may involve visible floating fish parts and oil, and attract scavenger birds and produce malodorous conditions.

Many of these problems are associated with disposal site areas with limited tidal or current flushing. Areas with strong tidal or current movement are able to disperse relatively large amounts of waste material as compared to areas where water movement is slow. No mention was made of any potential attraction of sharks to the waste areas.

In a study of ecological changes in Los Angeles - Long Beach Harbors, Soule and Oguri (1979) concluded that following the intensive control of toxic wastes in 1970, the formerly depauperate harbor experienced an enormous increase in species, higher taxa, and populations unprecedented in the area, in the period from 1971 to 1974. By far the greatest impact, they said, appears to have occurred when DAF and other pretreatment methods were installed in the canneries in 1974-1975. "It is now (1979) apparent that the harbor has been converted from the richest and most diverse soft-bottom community on the southern California coast to a less productive environment. The loss of food resources previously contained in the effluents has resulted in large order net reductions of organisms that fed directly or indirectly on the wastes."

Champ et al. (1981 and 1981a) state that fish processing plants located on islands in the tropics have limited land available for land treatment, high rainfall, poor soil percolation, a limited pet food market (high costs of shipment and small local pet population) and a low consumer preference for fish meal (because of high availability of local fishery resources). The impacts from ocean disposal of fish wastes, they said, can be: (1) high oxygen demand on receiving waters, (2) visible surface slick, (3) turbidity plume, (4) organic enrichment, and (5) the attractant of undesirable predator

species (i.e., sharks). "It will be difficult to predict or detect the effect of ocean disposal in deep waters. The attraction and possible retention of large numbers of sharks in a given area should be expected. The turbidity plume or eutrophication caused by nutrient enrichment can be very deleterious to coral reefs. However, these impacts, except for the sharks, can be reduced by: (1) the selection of a dumpsite, (2) determining the loading-assimilative capacity of the dumpsite ecosystem, and (3) determination of proper discharge rate. Monitoring programmes will be necessary for the detection and early warning that an alteration of the ecosystem is occurring in time to prevent irreversible deterioration." No citation, observation, or other information was presented to substantiate the statement regarding the potential attraction of sharks to fish waste disposal sites. No dumpsites are designated by EPA near coral reef communities.

Norton and Champ (1987) identified principal factors they believed to influence the effect of dumping of a particular sludge; such include:

- quantity dumped
- physical and chemical characteristics of the sludge
- method and rate of dumping
- water depth
- tidal currents
- wave-induced currents
- thermoclines or pycnoclines as barriers to dispersion
- sediment characteristics
- shelter from storms
- degree of natural sedimentation

Perhaps the most important of these are the extent of water movement at the seabed, and the nature of the sediments. The reports noted that if the objective is to aid dispersion, then discharge should be into the wake of a moving vessel where dilution is rapid enough to avoid flocculation of sludge

particles. Kolf (1985) notes that the initial dilution factors for pipeline diffusers can be somewhat more than 100, but dilution factors for barge dispersal are commonly near 1,000.

Only two field studies of ocean disposal of fish processing wastes have been made, and these were by Soule and Oguri (1982, 1983). The 1983 study was off Pago Pago, American Samoa, where water depth was 1200 meters, and the 1982 study was off Los Angeles, CA. In the 1983 study, the report states:

- No evidence of deleterious environmental stress was observed during ocean disposal monitoring.
- Although a visible surface plume remained for several hours following fish waste dumping, it did not appear as an oily slick but formed a suspensate of fine particulates which showed as a light blue-turquoise patch on the cobalt, deep sea waters.
- Dissolved oxygen did not fall below 5.5 mg/l.
- No sharks were sighted during disposal; it is believe that the waste particles in the sludge are too small to serve as an attractant.

At American Samoa, the sludge disposal vessel had a capacity of approximately 41,000 gallons. By calculation, if it is assumed that 40,000 gallons of sludge were dumped with a BOD of 150,000 mg/l in a receiving water with a DO of 6.0 mg/l, that combination of volume and BOD would consume the DO in 19 million 55-gallon drums of seawater or in a square mile of ocean 270 feet deep, if there were no introduction of DO into the water. Obviously, such an assumption is erroneous; much oxygen is introduced with surface turbulence and some oxygen is introduced through the activity of marine algae, although the phytoplankton population in Samoan waters is not high. During the study addressed by the 1983 report, DO never decreased below 5.5 mg/l; generally the maximum recorded was between 6.0 and 7.0 mg/l. The decline in DO that occurred following dumping was recovered in two hours.

Ammonia concentrations showed statistically significant increases during dumping to a depth of 3 meters; at 6 and 10 meters there was no significant difference from control concentrations. Ammonia principally was in the floatable waste fraction. Surface NH_3 concentrations were recorded in mg/l at 0.095, 0.119, 0.21, 0.33, and 0.41, for example.

There was good correlation between ammonia concentrations and BOD. The BOD at the surface was as high as 15.47 mg/l for one sample, but generally the higher BOD concentrations were 10.0 mg/l or lower.

The Soule and Oguri (1982) report describes testing following research oriented fish waste dumping. When brine and bail water alone were dumped, there was no discernable depression of DO. DAF sludge was included in the test dump loads in December, 1981, through March, 1982. In December a 0.4 mg/l drop in DO was measured in the first 7 minutes of disposal, from 8.6 to 8.2 mg/l. In February, the DO dropped from 8.4 mg/l to 6.7 mg/l but rebounded to 7.6 mg/l by the end of the dumping period. In March, there was a DO depression from 7.4 mg/l to 5.8 mg/l after 30 minutes, but there was recovery to 6.9 mg/l after 49 minutes from dumping.

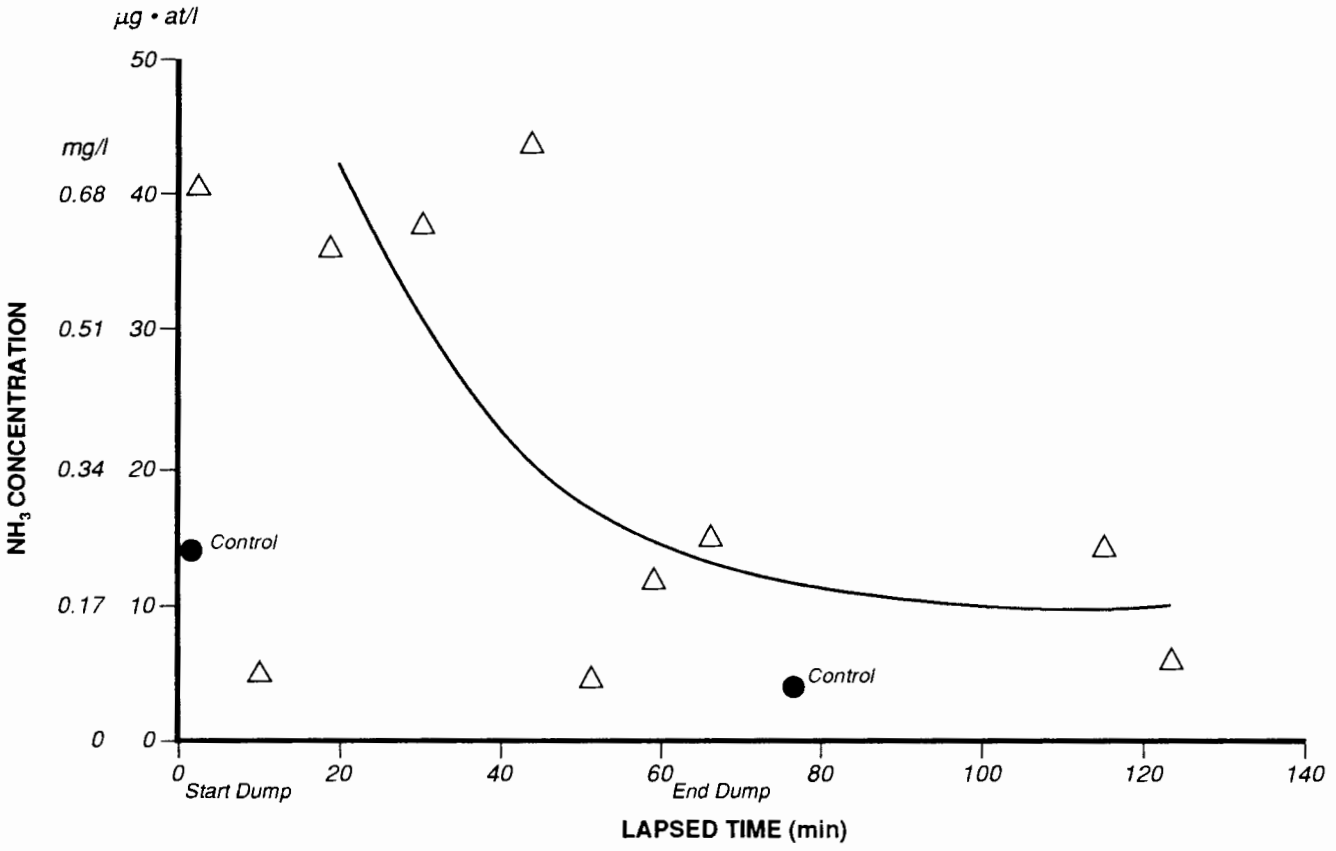
The ammonia, NH_3, concentrations ranged from 0.17 mg/l to 0.76 mg/l, which occurred in surface waters during dumping. There were some elevated values at 3, 6, and 10 meters, but most were within the range of variation and lacked statistical significance. The surface ammonia characteristics are shown in the figure on the following page.

The concentrations of ammonia-N in ocean waters are reported to range from 0.35 to 3.5 ug-at/l (microgram atoms per liter) or 0.006 to 0.06 mg/l (Sverdrup et al., 1946). Mean values for coastal waters off Los Angeles Harbor ranged from 0.70 ug-at/l in the spring of 1978 to 1.9 ug-at/l in the winter (Soule and Oguri, 1980). Background levels at control sites in American Samoa were slightly higher than those off Los Angeles, ranging from 1.6 to 4.9 ug-at/l.

Results of laboratory tests with fish wastes and sea water showed that ammonia persisted for 5 to 6 hours at levels between 40 and 60 ug-at/l (Soule and Oguri, 1984). This was followed by a precipitous decline to near zero. Soule and Oguri (1986) concluded from laboratory experiments that the coincidence of data for decline in DO and for ammonia-N suggests that the principal oxygen demand imposed by the waste is due to the degradation of ammonia by aerobic microheterotrophic bacteria.

No evidence of attraction of sharks or other undesirable fish species was found as a result of the experimental dumping, nor was there evidence of toxicity to resident or transient biota.

Both Soule and Oguri (1982 and 1983) reports describe laboratory experiments with DAF sludge. A column 14 feet high with a 2.5 inch ID was filled with seawater. Two-hundred ml of 33 percent sludge an 67 percent filtered seawater were added near the surface with a syringe. Samples were collected at various depths in the column through syringe ports or from a sediment cup at the base



Ammonia Levels In The Dump Plume And In The Post-dump Period On 5 February 1982; Hypothetical Curve. *(μg • at NH_3/l = 0.017mg NH_3/l)*

Taken from Soule and Oguri, 1982.

of the column in a 30 minute test and 120 minute test. The waste column was observed to separate into three distinct zones; a surface zone of floating material about 3.0 cm thick, a mixing zone with high turbidity, and a clear zone comprising about 75 percent of the water column through which discrete particles could be seen sinking toward the bottom.

At the end of 30 minutes, 92 percent of the recovered material was in the surface layer, and 0.5 percent was in the bottom sediment cup. At the end of the 120 minute test, 72.3 percent of the recovered material was in the surface zone and 7.1 percent was in the sediment cup. Ammonia analyses indicated that 97.9 percent of the recovered ammonia was in the surface layer in the 30 minute test, and 61.6 percent remained in the surface layer after 120 minutes. In both cases, the clear area showed less than 1.0 percent of the recovered ammonia and the remainder was in the mixing layer. The tests indicated that less than 10 percent of the material sank in 120 minutes.

In the previous section of this report, it was calculated that aluminum could be present in DAF sludge at a concentration of 27 mg/l; the reported concentration by Soule and Oguri ranged from 29 to 514 mg/l. DAF sludge had an LC_{50} to mysid shrimp of 400 mg/l.

Aluminum is amphoteric with minimum solubility at pH 5.5. Solubility increases as pH increases and as pH decreases. Thomas (1915) reported that aluminum as aluminum sulfate was toxic to mummichogs in 36 hours at 2.2 mg/l. More recent tests with fish showed aluminum as aluminum sulfate to have a brook trout 96-hour LC_{50} of 3.6 mg/l (Decker and Menendez, 1974); aluminum as aluminum chloride had a rainbow trout 72-hour LC_{50} of 5.2 mg/l (Freeman

and Everhart, 1971), and had a carp 48-hour LC_{50} of 4.0 mg/l (Muramoto, 1981). Jones (1939) published data indicating that aluminum as aluminum nitrate was lethal to the three-spine stickleback at 0.1 mg/l in 96 hours. Other tests show less sensitivity, e.g., no toxicity in 10 days to rainbow trout at 200 mg/l Al as Al_2So_4 (Hunter et al., 1980). The threshold concentration of aluminum sulfate for immobilization of Daphnia magna in Lake Erie water was found to be 106 mg/l (Anderson, 1944). No comparable data are available for salt water organisms. Although it would not be expected that aluminum would pose a problem to the ocean environment, it would be expected to impact test organisms within the close confines of a bioassay test chamber.

In testing pilot plant additional treatment of secondary treatment effluents from textile mills with three-species freshwater bioassays (fathead minnow, daphnid, and an alga), it was found that tertiary treatment systems employing alum or iron coagulation, in which residual concentrations of alum were greater than 9.0 mg/l Al or of iron were greater than 6.0 mg/l Fe, generally increased the toxicity of the waste-water. Likewise, coagulation with cationic polymers appeared to be toxic to freshwater algae (Rawlings, 1982).

Results of bioassay testing with the Microtox Toxicity Analyzer System* were reported to be "surprisingly consistent" with traditional bioassay results (Soule and Oguri, 1986). The System uses luminescent bacteria, and such a bioassay can be completed in 15 to 20 minutes. Before such a system is used as a means of predicting toxicity, however, the test should be performed with

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* Manufactured by Microbics Corporation, Carlsbad, CA

a reference toxicant obtained from EPA and in conjunction with a variety of organisms, including mysid shrimp, for example, to verify relative sensitivity of the different test organisms to a particular waste.

MONITORING PROGRAM

Environmental effects of a virtually organic and decomposable material discharged into ocean waters are controlled principally by conditions at the disposal site; such controlling conditions include water currents, depth, and the presence of a thermocline or pycnocline. These variables, likewise, would guide the degree of intensity of a monitoring program. Indeed, these variables may preclude the development of a generic monitoring program for all potential disposal sites.

The Marine Protection, Research, and Sanctuaries Act of 1972, as amended, provides guidance to the development of a monitoring program when it states in section 102 that the Administrator may issue permits for ocean disposal, except for certain specified types of wastes, when it is determined that such dumping will not unreasonably degrade or endanger human health, welfare, or amenities, or the marine environment, ecological systems, or economic potentialities, and in so far as the inviolability of applicable water quality standards is maintained.

Regulations were promulgated pursuant to the 1972 Act, and it is to these that one must turn to establish the fundamentals of a monitoring program. For example, following four hours after dumping, the DO may not be depressed by more than 25 percent below the normal anticipated ambient conditions in the disposal area at the time of dumping (40 CFR 227.7(e)). In studies conducted

to date there was no problem in meeting this requirement with fish wastes. The primary purpose of the monitoring program is to evaluate the impact of disposal on the marine environment by referencing the monitoring results to a set of baseline conditions (40 CFR 228.9(a)).

Sampling shall be done within the dump site itself and in the contiguous area. Sufficient control stations outside a disposal site shall be occupied to characterize the control area environment at least as well as the disposal site itself. Where there are known persistent currents, sampling in contiguous areas shall include at least two stations downcurrent of the dump site, and at least two stations upcurrent of the site (40 CFR 228.13(c)). 40 CFR 228.13 provides monitoring guidelines as opposed to monitoring requirements. Specifically included at all stations are measurements of temperature, DO, salinity, suspended solids, turbidity, TOC, pH, inorganic nutrients and chlorophyl a (40 CFR 228.13(d)(1)).

At one station near the center of the disposal site, samples of the water column should be taken for the analysis of the following parameters: mercury, cadmium, copper, chromium, zinc, lead, arsenic, selenium, vanadium, beryllium, nickel, pesticides, petroleum hydrocarbons and persistent organohalogens. These samples may be preserved for subsequent analysis by or under the direct supervision of EPA laboratories in accordance with the approved plan of study (40 CFR 228.13(d)(1)(i)).

Samples of the bottom should be taken for both sediment composition and structure, and to determine the nature and number of benthic biota (40 CFR 228.13(e)(1)). The number of required replicate samples per station is

specified as 3 for cores, 5 for grabs, 3 for dredge, and a 20-minute tow for trawl studies.

Fundamental to a monitoring program is a characterization of wastes to be ocean disposed. Characteristics of the wastes should be determined prior to ocean disposal by monthly analysis of a composite sample pooled from three replicate samples that are selected to represent the nature and composition of the fish waste; analyses should include:

- Bulk density
- pH
- Biochemical Oxygen Demand (BOD)
- Total Suspended Solids (TSS)
- Total Volatile Solids (TVS)
- Total nitrogen
- Ammonia-N
- Total phosphorus
- Oil and grease
- Aluminum
- Cadmium
- Mercury

For sample collection, holding, and analyses, see EPA 1987 and 40 CFR 136.

Acute toxicity tests of the DAF sludge should be performed quarterly using three-species bioassays involving a planktonic copepod, mysid shrimp, and appropriate fish from Table 1 of EPA, 1985. At the beginning of each series of tests a sample should be collected from the test solution prepared for each dilution tested, and the samples should be analyzed for temperature, DO, BOD, pH, salinity, ammonia-N, and aluminum.

During the monitoring of a fish waste disposal operation, the surface plume may be tracked by setting drogues near the surface and at three meters depth

at the time that dumping begins. Drogues should be tracked from their release until four hours after dumping has ceased. Surface drogues may be influenced by winds. The use of drogues is discussed in EPA, 1982. Visual observations and surface water ammonia analyses may be incorporated into surface plume tracking. The use of a transmissometer may be a viable alternative to either of the above for surface plume tracking.

Recognizing the necessity of identifying the extent and tracking of the surface plume in order to attain maximum efficiency in sampling station location, there are several techniques that are useful to achieve this objective. From recorded field studies, it is apparent that visual observation of the plume or floating waste solids is a useful but imprecise tool. Visible evidence should be confirmed by a quantifiable technique. A transmissometer measures the ability of a source of light to pass through a column of water. Solid particles in waste material would impede the passage of light; thus, the use of a transmissometer would measure the relative concentration of solid particles in the water column. The floating portion of processed fish wastes would be expected to have an abundance of waste particles, and these could be tracked with reasonable clarity through transmissivity readings.

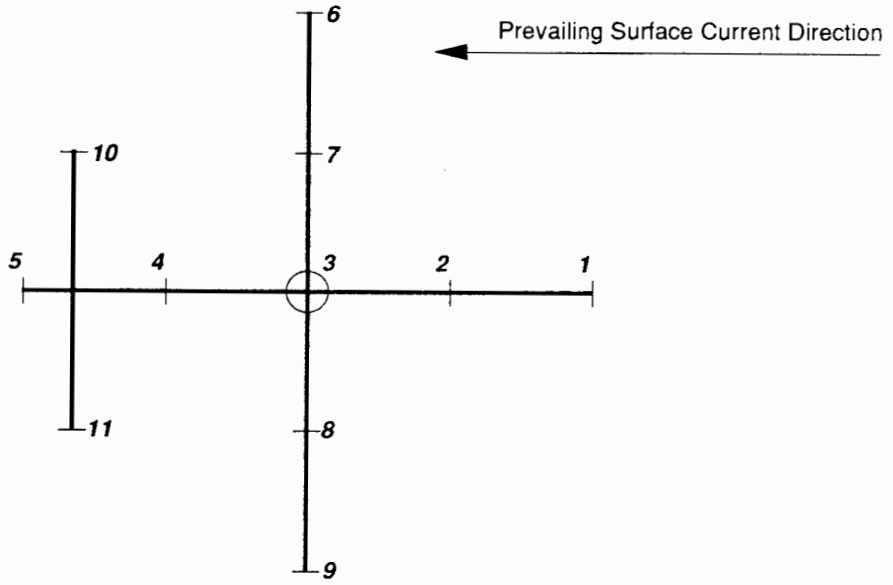
Processed fish wastes have elevated ammonia nitrogen concentrations; generally, there is a 10- to 100-fold increase in the ammonia concentration in the oceanic dump of processed fish wastes over that found in ambient oceanic water. The elevated ammonia concentrations persist for five to six hours following fish waste dumping. Such elevated ammonia concentrations could be identified with the use of an ammonia determining probe, and the surface plume

could be characterized through use of ammonia as a tracer. Cost of an ammonia probe would be expected to be somewhat less than the cost of a transmissometer but effective use of such a probe would depend upon the presence of measurable ammonia associated with the waste material. Certainly, prudence would dictate that backup equipment be available during the field testing in the event of instrument failure. Drogues, also, have been used to track currents that are associated with waste plumes. Drogues generally are drogue-buoy systems consisting of a small marker buoy that is tracked at the surface and a larger submerged drogue portion set at a desired depth and supported by a connecting line between the two. Drogues are intended to passively drift with the currents at a specified depth. A number of drogues need to be released together. They may be influenced to varying degree by the surface wind and the tracking and recovery sometimes can become resource intensive.

Monitoring at the dump site should be coordinated with a fish waste dumping operation and should be scheduled before, during, and until four hours after the dumping or until ambient quality conditions are reached, if sooner. In the establishment of sample collection locations and in the collection of samples, the positioning of the vessel is a vital consideration. Vessel positioning methods have been comprehensively examined in EPA, 1987a.

Water column collection locations, assuming a designated disposal site of 1.5 nautical miles in diameter, should be oriented toward following the surface plume of floatable materials. Three sampling configuration options are presented. The first two options could be considered as research sampling

programs. The last sampling configuration is a less complex monitoring program. One station configuration could be:



where the distance between 1 and 3 is 1.5 nautical miles, location 3 is the dumpsite area, and locations 1 and 2 would be upcurrent from the dumpsite.

Locations 1, 3, and 5 should be sampled at 3 meters prior to the disposal operation to establish ambient quality, location 3 should be sampled following the sighting of a visible plume from the dumping operation taking place in that immediate area, and locations outward from location 3 should be sampled in an order indicated by visible surface plume position and drogue sightings or transmissivity. Locations 1, 6, 9, 10, 11, and 5 are at the boundary of the designated dump site when the site has a diameter of 1.5 nautical miles (2.78 km).

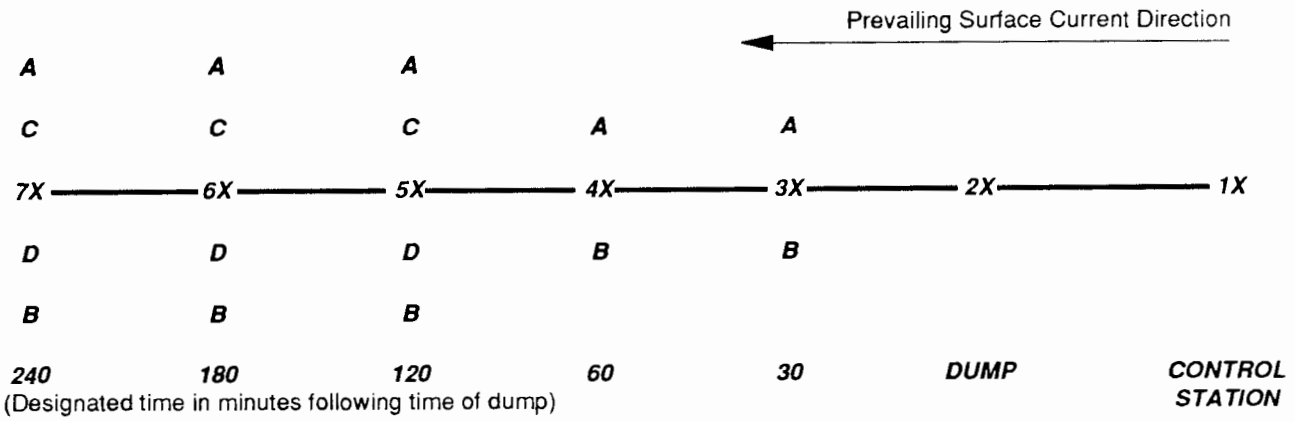
Water column sampling depths should be near-surface or within 10 cm of surface and at 3, 10, and 20 meters depths. A six meter sampling depth could be included as an alternative.

Constituents analyzed in samples from the water column should include:

Temperature
pH
Salinity
Dissolved Oxygen (DO)
Ammonia-N
Transmissivity

There is strong evidence, presented earlier, that ammonia-N is an excellent vehicle for surface plume tracking; there is evidence, also, that it would not need to be analyzed at depths greater than 10 meters. There is no apparent reason to include BOD as a water column test; the DO measurement will indicate direct environmental impact that may be attributable to BOD present. Total organic carbon analysis would not appear to contribute to our present understanding of fish wastes, nor would it contribute to expectations regarding environmental effects. Where there is a likelihood of marine phytoplankton excessive development, certainly strong consideration should be given to adding phosphorus and chlorophyll a to the water column monitoring list. The major form of inorganic nitrogen from this waste source is ammonia-N, which already is on the monitoring list. The surface floating plume, as indicated earlier, should be tracked visually with verification by ammonia-N analyses or transmissivity.

An alternative sampling scheme could be:



Orientation Of Sample Stations (Top View) Relative To The Visual Plume Centerline At The Time Of Sampling.

where Station 1X is 1.0 nautical mile (1.85 Km) upcurrent from Station 2X and is used as the control.

Station 2X is the center of the dumping operation, and should be sampled immediately after dumping begins.

Station 3X should be sampled 30 minutes after Station 2X, with a transmittance profile at the visual plume centerline. Stations 3A and 3B are sampled as soon as possible after 3, with the 3A profile 90^{o} and the 3B profile 270^{o} relative to Station 3X. Both 3A and 3B shall be within the plume 20 feet from the edge.

Station 4X is sampled 60 minutes after Station 2X, with a transmittance profile at the visual plume centerline. Stations 4A and 4B are sampled in the same manner as Stations 3A and 3B above.

Station 5X is sampled 120 minutes after Station 2X, with a transmittance profile at the visual plume centerline. Stations 5A and 5B are sampled in the same manner as Stations 3A and 3B above. Stations 5C and 5D are sampled as soon as possible after Station 5B. Stations 5C and 5D are aligned perpendicular to the centerline of the plume and one-half the distance between 5A and 5X or 5B and 5X, respectively.

Station 6X is sampled 180 minutes after Station 2X, with a transmittance profile at the visual plume centerline. Stations 6A, 6B, 6C and 6D are sampled in the same manner as Stations 5A through 5D described above.

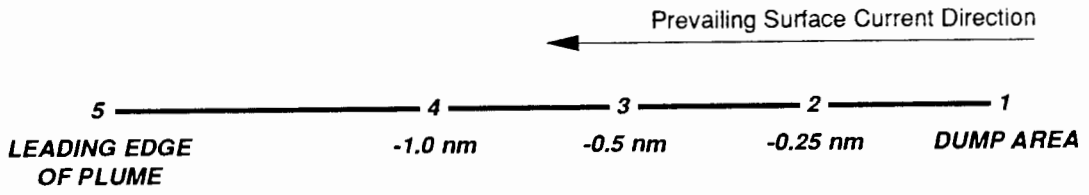
Station 7X is sampled 240 minutes after Station 2X, with a transmittance profile at the visual plume centerline. Stations 7A, 7B, 7C and 7D are sampled in the same manner as Stations 5A through 5D described above.

A transmittance profile should be taken to a depth of 10 meters at Stations 3, 4, 5, and 6 with measurements recorded at depths of 1, 3, and 10 meters. Transmittance profiles should be measured to a depth of 20 meters at Stations 1, 2, and 7. Exact locations and time of sampling of each of the profiles to the 90° or 270° of the centerline at each station to be determined by using the "best professional judgment" of the Principal Investigator on the monitoring vessel.

Current speed and direction should be determined at Stations 1X, 2X, and 7X by using an appropriate profiling current meter on each sampling cruise. Current speed and direction should be measured and recorded at the following depths: 1, 3, 10, and 20 meters.

On each sampling cruise, a water column profile to a depth of 20 meters for DO, pH, transmissivity, and Secchi disk depth should be made at Stations 1X, 2X, and 7X. Measurements should be taken at 1, 3, 10, and 20 meters. Total suspended solids, total volatile suspended solids, total phosphorus and total nitrogen and ammonia analyses on samples from these profiles could be made. Monitoring should be conducted monthly.

Another alternative sampling scheme could be:



Orientation Of Sample Stations (Top View) In The Middle Of The Discharge Plume Visually Identified At The Time Of Sampling.

Each sampling station is positioned as close as possible to the middle of the discharge plume and Station 1 is the center of the dumping operation.

Station 2 is 0.25 nautical miles (nm) down-current from Station 1.

Station 3 is 0.5 nm down-current from Station 1.

Station 4 is 1.0 nm down-current from Station 1.

Station 5 is at the leading edge of the discharge plume.

Control samples should be taken at Station 1 prior to dumping activities. Station 1 should be sampled again at a point within the plume immediately

after discharge operations cease. Stations 2 through 5 should be sampled consecutively at intervals to allow efficient sampling of the discharge plume. Samples should be taken at depths of 1, 3, and 10 meters at the middle of the plume as visually identified. Analyses of samples should include TSS, total volatile suspended solids, total phosphorus, total nitrogen, O&G, ammonia and pH. Samples should be collected and analyzed monthly.

Should water quality standards be applicable in the dump site area, the constituents identified therein would need to be incorporated into the analytical program, particularly in the period of four hours after dumping, which is identified as a time after which initial mixing has occurred (40 CFR 227.29(b)(1)). Throughout the sampling program, the regulatory purpose of a monitoring program, as specified in 40 CFR 228.9(a), i.e., to evaluate the impact of disposal on the marine environment by referencing the monitoring results to a set of baseline conditions, should be foremost in mind. Thus, it is believed that flexibility should be allowed in specifying sampling locations. As with most environmental investigations, the judgment of the on-site investigator related to particular field conditions at a specific time should take precedent over a pre-study developed study plan with sampling locations specifically identified.

A sample should be collected to meet the guidance of 40 CFR 228.13(d)(1)(i).

Because fish wastes are, in substantial part, floatable, and are of decomposable organic material with a high degree of volatile suspended solids, the above described monitoring program, conducted for a minimum of one week two times per year, should suffice for waters exceeding 200 meters in depth.

Modifications may need to be made in the program depending upon local dump site conditions or where dump sites are located in waters of less than 200 meters. Such modifications include the following:

- The need to include phosphorus and chlorophyll a in the list of analyses in waters that may be subject to excessive marine phytoplankton development has been mentioned. Chlorophyll a methodology is presented in EPA, 1987.

- How much of the dumped material is settled through the water column? What area of bottom does settling affect? The answers to such questions would be enhanced with the use of sediment traps. Sediment traps have been recommended for determining sewage sludge flux rates and depositional zones at the 106-mile ocean disposal site (O'Conner et al., 1985).

- Where water depths are suitable for benthic sampling, storage consideration should be given to an assessment of the marine macrobenthos. Such an assessment should demonstrate effects of any solids reaching the disposal site bottom area. The null hypothesis to be tested assumes no significant difference in biotic conditions between control and presumably stressed sites (Swartz, 1978). Swartz (1978) recommends that five 0.1 m^2 Smith-McIntyre grabs should be taken at each station and cruises should be conducted at least once every three months. The Reincke box corer used by Soule and Oguri (1986) would serve a dual purpose in sediment analyses; it samples 0.06 m^2 (0.67 ft^2) to a depth of 61 cm. It would collect sufficient sample for macro-benthos assessment, and it would provide a sediment core for sediment analysis. Procedures for sample management are provided in EPA, 1987. Tetra Tech (1985) concludes that number of species per unit area, number of individuals per unit area, dominance, abundance of pollution sensitive species, and abundance of opportunistic and pollution tolerant species are the most informative measurements of macro-benthos community structure.

- Where phytoplankton sampling may be required, e.g., water with eutrophic propensities, Stofan and Grant (1978) have provided details of sample collection and management. They conclude that chlorophyll a estimates of standing stock may yield pertinent correlative information with identification, enumeration, and productivity measurements and, thereby, contribute to the comprehensive phytoplankton community survey. This, however, may be an added cost burden that would impact ocean disposal decisions.

Costs of ocean monitoring are significant. Some selected items with approximate costs are listed below:

- Martek Electronic Probe (Temperature, salinity, pH and DO)	$ 7,500	(Naumann, 1987)
- Martek Transmissometer	$ 7,000	(Naumann, 1987)
- Current meter	$ 13,000	(Naumann, 1987)
- Theodolite navigational instrument	$ 700 to 16,000	(EPA, 1987)
- Electronic distance measurement instrument	$ 5000 to 20,000	(EPA, 1987)
- Orion ammonia probe	$ 450	(Avery, 1987)
- Boat rental per day	$ 600	(Soule, 1987)
- Sampling crew, 5 to 6 persons per day		(Soule, 1987)
- Sediment traps (stream), estimated	$ 5,000 to 10,000	(Wastler, 1987)

Obviously, monitoring costs are, in part, associated with the remoteness of the area to be monitored; remote areas pose difficult logistical problems.

PLUME MODELING

There is no mathematical model currently that can be used to predict the settleable plume and sediment disposition of the ocean bottom from the dumping of fish wastes. A sum of $10,000 has been set aside for adaptation of an existing model to conditions surrounding the ocean disposal of these wastes (Naumann, 1987).

In developing a model for the 106-mile sewage sludge ocean disposal site, Walker et al. (1987) assumed that initial dilution in the wake of a tanker eliminates any subsequent mixing resulting from density differences between waste and sea water; that constituents are completely conserved in the water

column with no transformation or degradation; and that all contaminants present in sludge are biologically available. O'Conner and Park (1982) indicated that disposal-barge-generated turbulence can be expected to mix wastes into volumes that are about 2.5 times the barge's width, three times the barge's draft, and as long as the dumping track. Such plumes widen, on the average, at a rate consistent with a diffusion velocity of 1 cm/sec.

Koh and Change (1973) developed a mathematical model for barge disposal of wastes, particularly dredged material. The Corps of Engineers, at their Waterways Experiment Station, modified the Koh and Chang concept into three models applicable for computing the fate of dredged material disposal: the continuous discharge model, the instantaneous dump model, and a stationary hopper dredge model (Johnson, 1987). The closest correlation between these models and the disposal of fish wastes would be the one designed to compute the movement of material in a continuous fashion at a constant discharge rate. All three models require that the disposed material be separated into various fractions with a settling velocity specified for each fraction. All models assume that the bulk density of the disposed material is greater than that of seawater. None of the models takes into consideration a discharge of material into the wake of a moving barge.

In all three models, the behavior of the material is assumed to be separated into three phases: convection descent, during which the dump cloud falls under the influence of gravity; dynamic collapse, occurring when the descending cloud or jet either impacts the bottom or arrives at a level of neutral buoyancy where descent is retarded and horizontal spreading dominates; and passive transport-dispersion, commencing when the material transport and

spreading are determined more by ambient currents and turbulence than by dynamics of the disposal operation.

Dredged material is composed of solid fractions, a fluid component, and perhaps a conservative chemical constituent. For each solid fraction, its concentration by volume, specific gravity, fall velocity, void ratio after deposit on the bottom, and an indicator as to whether or not the fraction is cohesive must be entered into the model. To trace a conservative chemical constituent, its initial concentration and a background concentration must be given. Certain disposal operations data must be entered. There are 15 coefficients in the model.

Johnson (1978a) did not believe that the above described models would apply to fish waste disposal principally on the grounds that the models assume a material density that is greater than one and that none of the models provides for disposal into the wake of a vessel for instant mixing. In developing these models, the discharge into the vessel's wake was deleted, which changed completely how the long-term transport diffusion concept was handled.

Teeter (1987), who has worked with these models and the initial Koh-Chang 1973 model while with EPA in the Corvallis Laboratory, cautioned that a model not designed for fish wastes disposal would take considerable effort in modification before it would be a workable entity. From a verbal description of the physical composition of fish wastes, Teeter's perception was that the material would become widely dispersed before it reached the ocean floor, especially in 1200 meters of water. Paul (1987), who has been engaged in sewage sludge modeling, believed that the limits of an existing model would be exceeded if one were to use such for fish wastes.

Fish wastes have an average bulk density of 0.89 ± 0.08 gm/ml to 1.00 ± 0.02 gm/ml, which is 87 percent to 98 percent the density of sea water. That portion of the waste with a bulk density of less than one will float on the ocean surface at least until the entrained air bubbles dissipate. Knowing surface currents and the time that a particular portion of the waste remains floating would lead to a capability to predict the spread of the floating plume with the use of a diffusion equation. That portion of the waste with a bulk density greater than one will form a plume toward the ocean floor. The net effect of the waste is the sum of the floating and settling effects.

The earlier model (Koh and Chang, 1973) assumes that the waste is composed of a solid phase characterized by constituents with various densities and fall velocities and a liquid phase. This model is also separated into one where the discharge is from a bottom opening hopper barge, and one where the material is discharged through a nozzel under a moving barge, and one where the discharge is into the barge wake.

Three phases of dispersion were envisioned in the Koh-Chang model: (1) a convective phase, (2) a collapse phase, and (3) a long term diffusion phase. The convection descent phase is due to the assumed density difference between the mixed waste material and ocean water. Johnson (1978a) expressed the opinion that the best approach to model development might be to examine the Koh-Chang model to determine if the model would accommodate waste with an average bulk density of less than sea water, but where some particles therein are heavier than sea water.

All of the above described models were structured to accommodate dredged material, which is relatively easily fractionated into sand, silt, clay,

liquid, etc., and the respective fall velocities determined. For a model to be effectively used for fish wastes, more would have to be known about waste characteristics, e.g., the ability to fractionate the waste in regard to various solids and liquid and to determine a bulk density and fall velocity for each fraction. More would have to be known about a particular dump site and currents at various depths and temperature, as well as salinity gradients. Also, the model would have to be field tested and verified for a particular disposal site.

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Date: 8/14/96

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OPTIONAL FORM 41 (Rev. 7-76)
Prescribed by GSA
FPMR (41 CFR) 101-11.206

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

August 14, 1996

MEMORANDUM

SUBJECT: Request for Review of Three Cannery Bioassay Reports

TO: Vance Fong, Chief
Quality Assurance Section (P-3-2)

FROM: Pat Young Pat
American Samoa Program Manager
Office of Pacific Island Programs (E-4)

We would appreciate your staff's review of three bioassay reports conducted for the American Samoa canneries' NPDES and ocean disposal permits. The reports are as follows:

1. Joint Cannery Ocean Dumping Studies in American Samoa, CH2MHill & Glatzel & Associates, July 1996. (Note this report consists of three bioassay reports and ocean disposal model evaluation. We are requesting review of only the third bioassay study (June 1995), as the two prior studies were reviewed previously.)

2. Bioassay Testing of Effluent, February 1996 (Delayed Fall Sept/Oct. 1995) Sampling, CH2MHill and Glatzel & Associates, August 9, 1996.

3. Bioassay Testing of Effluent, March 1996 Sampling, CH2MHill and Glatzel & Associates, August 9, 1996.

Please call me if you or your staff have any questions regarding these reports. We would like to have these reports reviewed within the next four weeks if possible. Thanks for your help.

cc: Allan Ota, W-3-2

OPINAP FAX TRANSMISSION
USEPA Region 9
Office of Pacific Island and Native American Programs (E-4)
75 Hawthorne Street
San Francisco, CA 94105
FAX NO: (415) 744-1604
VERIFICATION NO: (415) 744-1599

DATE: July 7, 1995 PAGES (incl. cover): 1

TO: Kurt Kline
Advanced Biological Testing Inc.

FAX: 415/435-7882 Phone: 415/435-7878

SUBJECT: Bioassay Test of Cannery Waste on Bi-valve Larvae

FROM: Pat Young, American Samoa Program Manager
USEPA Region 9
Phone: (415) 744-1594

Amy Wagner discussed with me the problems you were having with spawning the mussel larvae necessary for conducting bioassay tests on the cannery waste, and whether you should continue with the tests even though the cannery waste sample is now over 10 days old. Although the sample has been stored properly and refrigerated, we are concerned that given its high organic content and the waste's tendency to increase its ammonia content over time, no meaningful comparison or correlation of results could be made among the results of bioassay tests conducted on mussel larvae using 10-day-old cannery waste and the results obtained with the sand dab and mysid using the fresh sample. Rather than having you conduct the entire series again with the three species using new samples, and given the unrealibility of the mussel spawning, we waive the requirement to conduct the bioassay test on the mussel larvae for this round of sampling.

Should you have any questions, please feel free to call me.

cc: Steve Costa, CH2MHill
Jim Cox, Van Camp Seafoods
Norman Wei, Star-Kist Samoa
Amy Wagner, EPA Lab
Alan Ota, EPA (W-3-3)
Sheila, Wiegman, ASEPA

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX LABORATORY
1337 S. 46TH STREET BLDG 201
RICHMOND, CA 94804-4698

AUG [illegible]

MEMORANDUM

SUBJECT: Review of Bioassay Testing of Starkist, Samoa, Inc. and VCS Samoa Packing High Strength

FROM: Amy Wagner
Laboratory Section (P-3-1)

THRU: Brenda Bettencourt, Chief "Original Signed By"
Laboratory Section (P-3-1)

TO: Pat Young
OPINAP (E-4)

✓ Allan Ota
Wetlands and Sediment Management Section (W-3-3)

At your request, I have reviewed "Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa." The following recommendations are based on the results of the first round of testing.

1. p. 11. The salinity of the *Mysidopsis bahia* tests were 25 ppt, presumably based on the salinity of the shipping water. An effort should be made to find a supplier that raises mysids in a salinity closer to that of the discharge site, between 30-35 ppt.

2. Appendix, p. 1. It is recommended that the water quality measurements pH, dissolved oxygen, and initial salinity be measured for all samples upon receipt.

3. Appendix, Table 10. The salinities of 26-28 ppt most likely caused the high mortality in controls with the sea urchin toxicity test. If necessary, brine adjustments should be used to increase the salinity of test samples to the test method requirements of 30 $\pm$ 2 ppt.

4. To reduce salinity elevation throughout the tests, an attempt should be made to cover test containers to reduce evaporation.

Based on the results of these tests, the following changes in the bioassay methods recommended by CH2M Hill in the cover memo are acceptable.

1. The series of the concentrations for toxicity tests can be reduced to 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, and 0.0625% instead of the suggested series.

2. *Mytilus edulis* can be used instead of *Strongylocentrotus purpuratus* as the third test organism. The oyster *Crassostrea virginica* may be substituted for the mussel test during the months when mussels cannot be spawned.

3. Aeration should be provided in the mussel test containers due to high biological oxygen demand of the effluent. In addition to a control with aeration, a control without aeration should be run. A t-test should be used to determine if the there is any significant effect of aeration.

Any questions on the comments can be addressed to me at (510) 412-2329.

cc: Jeff Rosenbloom, Chief
Wetlands and Sediment Management Section (W-3-3)

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Date 5/29

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

August 29, 1994

Steven L. Costa
Project Manager
CH2M Hill
P.O. Box 12681
Oakland, CA 94604-2681

Re: Comments on Bioassay Testing of Ocean Disposed High-Strength Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

Dear Steve:

We have reviewed the report of June 29, 1994 for the first of three rounds of bioassays of high-strength waste, as required by the canneries' ocean disposal permits. The report is based on two sampling events: the first was collected on February 16, 1994; and, a second sample was required and tested in March 1994, due to test failure of the echinoderms in the first sample. Your proposed changes to the study methods, as outlined in your memo of July 1, 1994, are acceptable. Enclosed is a memo from Amy Wagner of EPA's Laboratory Support Section, detailing the acceptable changes. Please call Amy at (510) 412-2329 if you have any questions on her comments.

We note that the second and third rounds of testing were scheduled for May and August 1994, and we would like to know if these tests were conducted as scheduled and, if not, the rescheduled dates, and when we can anticipate the reports on these bioassays. Please relay this information to Pat Young, American Samoa Program Manager, or if you have any questions, call her at (415) 744-1594.

Sincerely,

for M. J. Lee
Norman L. Lovelace, Chief
Office of Pacific Island and Native American Programs (E-4)

Enclosure

cc: Jim Cox, Van Camp Seafood Company
Norman Wei, StarKist Seafood Company
Tony Tausaga, American Samoa EPA
Sheila Wiegman, American Samoa EPA
Allan Ota, W-3-3
Amy Wagner, P-3-1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX LABORATORY
1337 S. 46TH STREET BLDG 201
RICHMOND, CA 94804-4698

AUG 2 2 1994

MEMORANDUM

SUBJECT: Review of Bioassay Testing of Starkist, Samoa, Inc. and VCS Samoa Packing High Strength

FROM: Amy Wagner
Laboratory Section (P-3-1)

THRU: Brenda Bettencourt, Chief
Laboratory Section (P-3-1)

TO: Pat Young
OPINAP (E-4)

Allan Ota
Wetlands and Sediment Management Section (W-3-3)

At your request, I have reviewed "Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa." The following recommendations are based on the results of the first round of testing.

1. p. 11. The salinity of the *Mysidopsis bahia* tests were 25 ppt, presumably based on the salinity of the shipping water. An effort should be made to find a supplier that raises mysids in a salinity closer to that of the discharge site, between 30-35 ppt.

2. Appendix, p. 1. It is recommended that the water quality measurements pH, dissolved oxygen, and initial salinity be measured for all samples upon receipt.

3. Appendix, Table 10. The salinities of 26-28 ppt most likely caused the high mortality in controls with the sea urchin toxicity test. If necessary, brine adjustments should be used to increase the salinity of test samples to the test method requirements of 30 $\pm$ 2 ppt.

4. To reduce salinity elevation throughout the tests, an attempt should be made to cover test containers to reduce evaporation.

Based on the results of these tests, the following changes in the bioassay methods recommended by CH2M Hill in the cover memo are acceptable.

1. The series of the concentrations for toxicity tests can be reduced to 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, and 0.0625% instead of the suggested series.

2. *Mytilus edulis* can be used instead of *Strongylocentrotus purpuratus* as the third test organism. The oyster *Crassostrea virginica* may be substituted for the mussel test during the months when mussels cannot be spawned.

3. Aeration should be provided in the mussel test containers due to high biological oxygen demand of the effluent. In addition to a control with aeration, a control without aeration should be run. A t-test should be used to determine if the there is any significant effect of aeration.

Any questions on the comments can be addressed to me at (510) 412-2329.

cc: Jeff Rosenbloom, Chief
Wetlands and Sediment Management Section (W-3-3)

ROUTING AND TRANSMITTAL SLIP

Date: 2/8/95

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REMARKS

Copy of bioassay results. Amy Wagner of the lab is reviewing w/in next 3 weeks.

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MEMORANDUM CH2MHILL

TO: Pat Young/USEPA

COPIES: Amy Wagner/USEPA (w/ attachments)
Norman Wei/StarKist Foods (w/attachments)
James Cox/Van Camp Seafood (w/attachments)
Sheila Wiegman/American Samoa EPA (w/attachments)
Kurt Kline/ABT (w/o attachments)

FROM: Steve Costa/CH2M HILL/SFO
Karen Glatzel/Glatzel & Associates

DATE: 26 January 1995

SUBJECT: **Bioassay Testing of High Strength Waste: Starkist Samoa, Inc. and VCS Samoa Packing**

PROJECT: OPE030702.DS.BT

Three sets of bioassay tests with high strength waste (HSW) are required by Special Condition 3.3.5 of Starkist Samoa's and VCS Samoa Packing's ocean dumping permits. The results of the second set of tests are presented in the attached: "*Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa*" prepared by Advanced Biological Testing Inc. (ABT), Tiburon, California, dated November 21, 1994 (Attachment No. 1). The second sampling was conducted on 20 October 1994 and sampling procedures are provided as Attachment No. 2.

Acute effluent bioassays were conducted on *Mysidopsis bahia* (mysid shrimp) juveniles, *Mytilus edulis* (blue mussel) larvae, and *Citharichthys stigmaeus* (speckled sanddab) juveniles using HSW collected separately from the Starkist Samoa and VCS Samoa Packing canneries in Pago Pago Harbor, American Samoa. The results of these bioassays are summarized in the table below. Test results from the first set of tests (16 February 1994 sampling) are included in the table for comparison.

After the first set of tests CH2M HILL and ABT recommended a number of changes to the HSW test protocol (Attachment No. 3). U.S. EPA's response to the recommendations is provided in Attachment No. 4. The recommendation for reducing the maximum concentrations of the samples was accepted by U.S. EPA and after consultation between ABT and EPA new test concentrations were established for the mysid, mussel, and sanddab tests of 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% as a volume dilution in 30 ppt seawater. The recommendation for dropping the urchin test was accepted by U.S. EPA. The mussel test was continued to investigate the effects of aeration as described below.

In the first test (2/94) it was determined that due to the high oxygen demand, including a high immediate oxygen demand, of the effluent all test containers required aeration

throughout the tests to maintain adequate oxygen concentrations. Aeration is standard protocol for bioassays on fish and invertebrates when oxygen levels fall below 40% of saturation, but is not standard protocol for bioassays on larval bivalves and echinoderms. Therefore, aerating the chambers containing *Mytilus edulis* may give problematic results.

In the second test (October 1994 sampling) gentle aeration was initiated on Day 0, and continued for the duration of the tests. To assess the effects of aeration, an aeration control for the mussel test was run simultaneously. No statistical differences were observed between aerated and unaerated controls. It is now recommended that this type of aeration continue to be used with the mussel test to determine if a permanent change in the protocols for these samples should be made regarding aeration.

After review of the test results, we suggest Amy Wagner contact Kurt Kline, Advanced Biological Testing Inc., directly at (415) 435-7878 to discuss any comments on the bioassay tests or the test protocols. Please contact Steve Costa, at (510) 251-2888 ext 2251, if there are any additional questions regarding this memo.

Summary of High Strength Waste Bioassay Results.

Test Organism	Endpoint	Starkist Samoa		VCS Samoa Packing	
		2/94	10/94	2/94	10/94
Citharichthys stigmaeus (sanddab)	LC_{50}	0.27%	0.35%	0.59%	0.37%
	NOEC	0.20%	0.25%	0.40%	0.25%
	LOEC	0.40%	0.50%	0.80%	0.50%
Mysidopsis bahia (mysid shrimp)	LC_{50}	0.12%	1.16%	0.59%	0.79%
	NOEC	0.05%	0.50%	0.05%	0.50%
	LOEC	0.10%	1.00%	0.10%	1.00%
Mytilus edulis (blue mussel)	LC_{50}	>1.20%	>2.0%	>1.20%	>0.20%
	IC_{50}	<0.08%	0.10%	<0.08%	0.18%
Strongylocentrotus pupuratus (urchin)[1]	LC_{50}	1.20%	-	1.20%	-
	IC_{50}	<0.08%	-	0.10%	-

[1] Urchin test not conducted in 10/94 test period as per direction from U.S. EPA.

ATTACHMENT I

Laboratory Report of Bioassay Results for

High Strength Waste Sampling

20 October 1994

RESULTS OF BIOASSAYS CONDUCTED ON TWO HIGH STRENGTH WASTE SAMPLES FROM THE VAN CAMP AND STARKIST TUNA CANNERIES IN AMERICAN SAMOA

Prepared for:

CH2M Hill California, Inc.
1111 Broadway
Oakland, CA 94607
Project # PDX 30702

Prepared by:

Advanced Biological Testing Inc.
98 Main St., # 419
Tiburon, Ca. 94920

November 21, 1994

Ref: 9309-3

1.0 INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted acute effluent bioassay testing on *Mysidopsis bahia*, *Mytilus edulis*, and *Citharichthys stigmaeus* using high strength wastes (HSW) collected separately from the Starkist (HSW-1) and Van Camp (HSW-2) tuna canneries in American Samoa. The study was run using methods generally specified in EPA 1991 and in a Sampling and Testing Plan submitted to the EPA.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.0 METHODS

2.1 EFFLUENT SAMPLING

The high strength wastes were sampled as composites on October 20, 1994 by personnel from the two canneries. Due to shipping and airline scheduling problems, frequently encountered in this region, the sample was received by the laboratory on October 24, 1994. A single gallon carboy was provided from each cannery and were labeled at ABT as HSW-1 (HSW-SKS Grab) and HSW-2 (Pipeline Sludge HS-W2, Van Camp). Samples were maintained in ice-filled coolers from the date of sampling until laboratory receipt. The samples were at 2-3°C upon receipt and were stored at 4°C until use.

2.2 SAMPLE PREPARATION AND TESTING METHODS

2.2.1 Testing on the speckled sanddab, *Citharichthys stigmaeus*

In agreement with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at six concentrations starting from 2.0% and dropping using a 50% dilution factor. The final concentrations were 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% as vol:vol dilutions in seawater. The diluent was filtered seawater from San Francisco Bay. The dilutions were brought up to the test temperature (17 ± 2°C) and aerated continuously. These effluents have an extremely high biological oxygen demand, therefore aeration was carried out from the beginning of the test.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 25, 12.5, 6.25, 3.1, and 1.6 mg/L in 30 ppt seawater in a 24 hour test.

The bioassays were carried out on juvenile *Citharichthys stigmaeus*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on October 24, 1994. The test conditions are summarized in Table 1. Five replicates of each concentration were tested with ten juvenile fish per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.2 Testing on the mysid, *Mysidopsis bahia*

In agreement with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at six concentrations starting from 2.0% and dropping using a 50% dilution factor. The final concentrations were 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% as vol:vol dilutions in seawater. The diluent was filtered seawater from San Francisco Bay The dilutions were brought up to the test temperature (16 ± 2°C) and aerated continuously.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 40, 20, 10, 5, 2.5 and 1.25 mg/L in 30 ppt seawater in a 96 hour test.

The first bioassay was carried out on 7-10 day old larval *Mysidopsis bahia*, supplied by Aquatox from Hot Springs, Arkansas. The animals were received at ABT on November 1, 1994. The test conditions for this test are summarized in Table 2. Five replicates of each concentration were tested with ten larval mysids per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.3 Bivalve Larval Bioassay

Test solutions used in the bioassays were prepared using San Francisco Bay seawater at 30 ppt in serial dilution (0.5) to create 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% test concentrations for the bioassays. The bivalve study was conducted under ASTM 1993 guidelines.

The reference toxicant for the bivalve larval bioassays was copper sulfate at test concentrations of 3.75, 7.5, 15, 30, and 60 µg/L.

The bivalve larvae survival and development test was run following methods in ASTM (1993). Bay mussels, *Mytilus edulis,* were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by heat shocking. Released gametes were placed in individual containers of filtered seawater and examined for viability. Gametes were mixed and allowed to fertilize for up to two hours, under gentle aeration. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 µm. Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to

an initial density of 15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at 16 ± 2°C under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0 and 48 hours; temperature was also recorded at 24 hours. Total ammonia in the 2% concentration was 3.6 mg/L at test initiation for HSW-1 and 6.1 mg/L for HSW-2. Ammonia was not measured on Day 2. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

Gentle aeration was initiated on Day 0, and continued for the duration of the tests. To assess the effects of aeration, an aeration control was run simultaneously. No statistical differences were observed between aerated and unaerated controls.

2.3 STATISTICAL ANALYSIS

At the conclusion of the testing, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and LOEC values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis. Data were evaluated statistically to estimate the LC50 and IC50 values for the tests using the Probit or Trimmed Spearman-Karber Method.

3.0 RESULTS

3.1 Initial Effluent Quality

The two High Strength Wastes were tested for basic water quality parameters upon receipt at the laboratory. HSW-1 had a dissolved oxygen level of 0.7 mg/L; a pH of 6.53; a salinity of 23.5 ppt; and a total ammonia level of 480 mg/L. HSW-2 had a dissolved oxygen level of 0.6 mg/L; a pH of 6.39; a salinity of 14.0 ppt; and a total ammonia level of 350 mg/L.

3.1 *Citharichthys stigmaeus*

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at 17 ± 2°C; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

The LC50 for HSW-1 was 0.35% based upon a Trimmed Spearman-Karber method. The majority of the observed toxicity again occurred in the first 24 hours. There was significant mortality at 2.0, 1.0, and 0.5% concentrations compared to the control at 96 hours. The NOEC was 0.25% and the LOEC was 0.5%

The LC50 for HSW-2 was 0.37% based upon a Trimmed Spearman-Karber method. The majority of the observed toxicity occurred in the first 24 hours. There was significant mortality at 2.0, 1.0, and 0.5% concentrations compared to the control at 96 hours. The NOEC was 0.25%, and the LOEC was 0.5%.

The reference toxicant test required the use of the Trimmed Spearman-Karber method and generated an LC50 of 3.9 mg/L, an NOEC of 3.1 mg/L, and an LOEC of 6.25 mg/L. This is the third reference toxicant test on *Citharichthys* at this laboratory, therefore no database has been established by this laboratory although the data has been consistent in the 3 - 4 mg/L range. The current laboratory mean is 3.92 mg/L.

3.2 *Mysidopsis bahia*

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at 17 ± 2°C; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

The LC50 for HSW-1 was 1.16%. At 96 hours, there was significant mortality at 2.0 and 1.0% concentrations compared to the control. The NOEC was 0.5% and the LOEC was 1.0%.

The LC50 for HSW-2 was 0.79%. again there was significant mortality at 96 hours in the 2.0 and 1.0% concentrations compared to the control. The NOEC was 0.5%, and the LOEC was 1.0%.

The reference toxicant test had an LC50 of 7.27 mg/L, with an NOEC of 1.25 mg/L and an LOEC of 2.5 mg/L. This is the third reference toxicant test on *Mysidopsis* at this laboratory, therefore no database has been established. The current mean is 13.5 mg/L.

3.3 Bivalve Larval Bioassay

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at 17 ± 2°C; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

Control survival was acceptable at 100% with 1.4% abnormal development. The LC50 for HSW-1 was >2.0%, while the LC50 for HSW-2 was 0.2%. The IC50 for HSW-1 was 0.1% and the IC50 for HSW-2 was 0.18%.

The LC50 (6.1 µg/L) for the copper sulfate reference toxicant test was within two standard deviations of the laboratory mean of 15.9 µg/L indicating normal to higher sensitivity of the test organisms.

3.5 AMMONIA MEASUREMENTS

Ammonia in both of the HSW was very high. When measured in a 25% dilution in seawater, ammonia levels ranged from 88 to 120 mg/L. When converted to the 100% concentration, the ammonia level would be above 350 - 450 mg/L. The un-ionized fraction as NH_3 would range from 17 to 24 mg/L at 100% concentration.

Advanced Biological Testing Inc.

TABLE 1

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Citharichthys stigmaeus* (U.S. EPA 1991)

Parameter	Data
Test Species	*Citharichthys stigmaeus*
Supplier	J. Brezina and Associates
Collection location	Tomales Bay
Date Acquired	10/25/94
Acclimation Time	24 hours
Acclimation Water	30 ppt seawater
Acclimation Temperature	12 ± 2°C
Age group	Juveniles, 3-5 cm TL
Sample Identification	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	One gallon
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	96 hour static acute, renewal at 48 hours
Test Dates	10/26/94 to 10/30/94
Control Water	San Francisco Bay seawater
Test Temperature	17 ± 2°C
Test Photoperiod	16 L : 8 D
Initial Salinity	31 ± 2 ppt
Test Chamber	10 L polyethylene chamber
Animals/Replicate	10 animals/replicate
Exposure Volume	5 L
Replicates/Treatment	5
Feeding	None
Deviations from procedures	None

TABLE 2

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Mysidopsis bahia* (U.S. EPA 1991)

Parameter	Data
Test Species	*Mysidopsis bahia*
Supplier	Aquatox, Arkansas
Date Acquired	11/1/94
Acclimation Time	None
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	7-10 day larvae
Sample Identification	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	11/1/94 to 11/5/94
Control Water	San Francisco Bay seawater
Test Temperature	18 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	30 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	None

TABLE 3

Bioassay Procedure And Organism Data For The 48 Hour Bioassay Using Larvae of *Mytilus edulis* (ASTM 1993)

Parameter	Data
Test Species	*Mytilus edulis*
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	10/25//94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
Sample Identification	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	One gallon
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute; static; 48 hours
Test Dates	10/25/94 to 10/27/94
Control Water	San Francisco Bay seawater
Test Temperature	16 ± 2°C
Test Photoperiod	16 L : 8 D
Salinity	32 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	3
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 4

SUMMARY OF RESULTS
FOR THE HIGH STRENGTH WASTE BIOASSAYS

Species	Test	Endpoint	HSW-1	HSW-2
Citharichthys stigmaeus	96 hr static	LC50	0.35%	0.37%
		NOEC	0.25%	0.25%
		LOEC	0.50%	0.50%
Mysidopsis bahia	96 hr static	LC50	1.16%	0.79%
		NOEC	0.50%	0.50%
		LOEC	1.00%	1.00%
Mytilus edulis	48 hr static	LC50	>2.0	0.20%
		IC50	0.10%	0.18%

Note:
HSW-1: Starkist
HSW-2: Van Camp

TABLE 5

SUMMARY OF RESULTS FOR THE REFERENCE TOXICANT TESTS

Citharichthys stigmaeus Concentration (mg/L)	SDS % Survival	LC50 (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Control	100.0	3.9	3.1	6.25
1.6	100.0			
3.1	83.3			
6.25	0.0*			
12.5	0.0*			
25	0.0*			

Lab LC50 = 3.92.

Mysidopsis bahia Concentration (mg/L)	SDS % Survival	LC50 (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Control	98.0	7.27	1.25	2.5
0.7	90.0			
1.25	90.0			
2.5	73.3*			
5	83.3*			
10	70.0*			
20	10.0*			
40	0.0*			

Lab LC50 = 13.52.

Bivalve larvae Concentration (µg/L)	Copper sulfate Mean Normal Larvae/mL	% Treatment Mortality	LC50 (µg/L)	(%) Abnormal
Initial Counts	23.5		6.1	
Control W/Air	23.5	NA		1.4
Control WO/Air	22.9	NA		3.8
3.75	19.0	6.4		1.8
7.5	2.3*	88.5		51.9
15	4.7*	76.7		100
30	0.0*	100.0		100
60	0.0*	100.0		100

* Statistically significant.

4.0 REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.

ASTM. 1993. Annual Book of Standards. Vol. 11.04. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve mollusca. E-724-89.

APPENDIX A

ANALYTICAL DATA

APPENDIX TABLE 1

SAMPLE WATER QUALITY

Sample	pH (units)	DO (mg/L)	Total NH3 (mg/L)	Initial Salinity (ppt)
HSW-1	6.53	0.7	480	23.5
HSW-2	6.39	0.6	350	14

APPENDIX TABLE 2

Mytilus edulis

WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST

Test Dates: 10/25-10/27/94

Concentration		Day 0				Day 1	Day 2			
(%)	Rep	pH	DO	°C	Sal	°C	pH	DO	°C	Sal
Control	1	8.06	8.8	16.7	32	16.2	8.00	8.8	16.9	32
W/Air	2					16.3	8.01	8.8	16.9	32
	3					16.2	8.02	8.6	16.9	32
Control	1	8.06	8.8	16.7	32	16.2	8.09	8.8	16.9	32
WO/Air	2					16.2	8.11	8.8	16.9	32
	3					16.2	8.13	8.8	16.9	32
HSW-1										
0.06	1	8.04	8.8	16.8	32	16.3	8.12	8.8	16.9	32
	2					16.2	8.09	8.7	16.9	32
	3					16.2	8.11	8.8	16.9	32
0.125	1	7.99	8.8	16.8	32	16.3	8.14	8.6	16.9	32
	2					16.2	8.08	8.6	16.9	33
	3					16.2	8.12	8.7	16.9	32
0.25	1	7.88	8.8	16.7	32	16.2	8.14	8.6	16.9	33
	2					16.2	8.12	8.6	16.9	32
	3					16.3	8.08	8.5	16.9	32
0.5	1	7.68	8.8	16.6	32	16.2	8.02	6.2	16.9	32
	2					16.2	7.75	6.0	16.9	32
	3					16.2	7.68	6.1	16.9	32
1	1	7.34	8.8	16.6	32	16.2	8.01	4.8	16.9	32
	2					16.3	8.00	4.9	16.9	32
	3					16.3	7.93	4.8	16.9	32
2	1	6.96	8.4	16.6	32	16.2	8.04	3.4	16.9	32
	2					16.2	7.99	3.2	16.9	32
	3					16.2	8.05	3.4	16.9	32
	Min	6.96	8.4	16.6	32	16.2	7.68	3.2	16.9	32
	Max	8.06	8.8	16.8	32	16.3	8.14	8.8	16.9	33

APPENDIX TABLE 2 (Cont'd)

Mytilus edulis

WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST

Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1	Day 2			
(%)	Rep	pH	DO	°C	Sal	°C	pH	DO	°C	Sal
HSW-2										
0.06	1	8.06	8.8	16.7	32	16.3	8.12	8.6	16.9	32
	2					16.3	8.15	8.5	16.9	32
	3					16.3	8.16	8.6	16.9	32
0.125	1	8.04	8.9	16.6	32	16.2	8.17	8.5	16.9	32
	2					16.2	8.17	8.5	16.8	32
	3					16.2	8.19	8.5	16.9	32
0.25	1	7.94	8.8	16.7	32	16.2	8.20	8.4	17.0	32
	2					16.2	8.19	8.5	16.9	32
	3					16.3	8.14	8.2	16.9	32
0.5	1	7.77	8.7	16.7	32	16.3	7.73	3.4	16.9	32
	2					16.3	8.11	7.8	16.9	32
	3					16.3	8.15	7.8	16.9	32
1	1	7.40	8.7	16.8	32	16.2	8.09	7.4	17.0	32
	2					16.2	8.19	7.6	16.9	32
	3					16.2	8.20	7.6	16.9	32
2	1	6.92	8.6	16.6	32	16.2	8.03	3.8	16.9	32
	2					16.2	8.03	4.8	16.9	32
	3					16.2	7.98	4.6	16.9	32
	Min	6.92	8.6	16.6	32	16.2	7.73	3.4	16.8	32
	Max	8.06	8.9	16.8	32	16.3	8.20	8.6	17.0	32

APPENDIX TABLE 3

Mytilus edulis

SUMMARY OF RESULTS FOR BIVALVE LARVAE HIGH STRENGTH WASTE BIOASSAY

Test Dates: 10/25-10/27/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	110		22.0			
	2	135		27.0			
	3	108		21.6			
	Mean			23.5			
Final Control W/Air	1	101	0	20.2		0.0	
	2	129	0	25.8		0.0	
	3	117	5	24.4		4.1	
	Mean			23.5	100.0	1.4	NA
Final Control WO/Air	1	104	5	21.8		4.6	
	2	109	3	22.4		2.7	
	3	118	5	24.6		4.1	
	Mean			22.9	100.0	3.8	NA
HSW-1							
0.06	1	82	12	18.8		12.8	
	2	89	14	20.6		13.6	
	3	78	15	18.6		16.1	
	Mean			19.3	93.4	14.2	4.8
0.125	1	23	72	19.0		75.8	
	2	18	58	15.2		76.3	
	3	20	71	18.2		78.0	
	Mean			17.5	84.4	76.7	14.0
0.25	1	3	82	17.0		96.5	
	2	1	77	15.6		98.7	
	3	3	85	17.6		96.6	
	Mean			16.7	80.8	97.3	17.6
0.5	1	0	85	17.0		100.0	
	2	0	93	18.6		100.0	
	3	0	81	16.2		100.0	
	Mean			17.3	83.4	100.0	14.9
1	1	0	89	17.8		100.0	
	2	0	94	18.8		100.0	
	3	0	97	19.4		100.0	
	Mean			18.7	90.2	100.0	8.0
2	1	0	95	19.0		100.0	
	2	0	96	19.2		100.0	
	3	0	87	17.4		100.0	
	Mean			18.5	89.5		8.7

APPENDIX TABLE 3 (Cont'd)

Mytilus edulis

SUMMARY OF RESULTS FOR BIVALVE LARVAE HIGH STRENGTH WASTE BIOASSAY

Test Dates: 10/25-10/27/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2							
0.06	1	102	3	21.0		2.9	
	2	87	2	17.8		2.2	
	3	117	3	24.0		2.5	
	Mean			20.9	100.0	2.5	0.0
0.125	1	67	13	16.0		16.3	
	2	61	12	14.6		16.4	
	3	52	12	12.8		18.8	
	Mean			14.5	69.9	17.1	28.7
0.25	1	0	38	7.6		100.0	
	2	0	27	5.4		100.0	
	3	0	33	6.6		100.0	
	Mean			6.5	31.6	100.0	67.8
0.5	1	0	27	5.4		100.0	
	2	0	27	5.4		100.0	
	3	0	27	5.4		100.0	
	Mean			5.4	26.1	100.0	73.4
1	1	0	36	7.2		100.0	
	2	0	39	7.8		100.0	
	3	0	31	6.2		100.0	
	Mean			7.1	34.1	100.0	65.2
2	1	0	37	7.4		100.0	
	2	0	31	6.2		100.0	
	3	0	36	7.2		100.0	
	Mean			6.9	33.5	100.0	65.8

APPENDIX TABLE 4

Mytilus edulis
WATER QUALITY MEASUREMENTS
FOR THE REFERENCE TOXICANT (C0PPER) TEST

Concentration		Day 0				Day 1	Day 2			
µg/L	Rep	pH	DO	°C	Sal	°C	pH	DO	°C	Sal
3.75	1	8.08	8.8	16.7	32	16.4	8.15	8.4	17.0	32
	2					16.4	8.13	8.5	16.9	32
	3					16.4	8.15	8.6	16.9	32
7.5	1	8.09	8.8	16.7	32	16.5	8.18	8.6	16.9	32
	2					16.4	8.18	8.4	16.9	32
	3					16.5	8.16	8.4	16.9	32
15	1	8.10	8.7	16.7	32	16.5	8.17	8.5	16.9	32
	2					16.5	8.18	8.5	17.0	32
	3					16.5	8.18	8.4	17.0	32
30	1	8.10	8.7	16.8	31	16.5	8.17	8.4	16.9	32
	2					16.5	8.17	8.4	16.9	32
	3					16.5	8.16	8.5	16.9	32
60	1	8.11	8.7	16.7	30	16.5	8.16	8.5	16.9	32
	2					16.4	8.17	8.6	16.9	32
	3					16.5	8.16	8.6	17.0	32
	Min	8.08	8.7	16.7	30	16.4	8.13	8.4	16.9	32
	Max	8.11	8.8	16.8	32	16.5	8.18	8.6	17.0	32

APPENDIX TABLE 5

Mytilus edulis
SUMMARY OF RESULTS FOR THE BIVALVE LARVAE REFERENCE TOXICANT (COPPER) BIOASSAY

Concentration (µg/L)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
3.75	1	90	2	18.4		2.2	
	2	97	1	19.6		1.0	
	3	93	2	19.0		2.1	
	Mean			19.0	91.8	1.8	6.4
7.5	1	4	5	1.8		55.6	
	2	6	7	2.6		53.8	
	3	7	6	2.6		46.2	
	Mean			2.3	11.3	51.9	88.5
15	1	0	27	5.4		100.0	
	2	0	21	4.2		100.0	
	3	0	23	4.6		100.0	
	Mean			4.7	22.9	100.0	76.7
30	1	0	0	0.0		100.0	
	2	0	0	0.0		100.0	
	3	0	0	0.0		100.0	
	Mean			0.0	0.0	100.0	100.0
60	1	0	0	0.0		100.0	
	2	0	0	0.0		100.0	
	3	0	0	0.0		100.0	
	Mean			0.0	0.0	100.0	100.0

APPENDIX TABLE 6

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	7.98	7.9	0.03	17.1	32	8.18	8.2		17.2	33	8.16	7.2	0.02	17.1	33	8.17	7.3	0.03	17.4	33	8.05	8.0	0.03	17.9	34
	2						8.23	8.1	0.03	17.0	33	8.23	7.2		16.5	33	8.22	7.2		17.1	33	8.14	8.0		17.7	34
	3						8.22	8.1		16.9	32	8.24	7.2		16.3	33	8.24	7.3		16.9	33	8.17	8.0		17.6	34
	4						8.22	8.4		16.6	33	8.24	7.2		16.2	33	8.24	7.4		16.8	33	8.18	8.1		17.5	34
	5						8.22	8.5		16.5	33	8.24	7.4		16.0	33	8.25	7.4		16.6	33	8.20	8.2		17.2	34
0.06	1	7.93	8.0	0.14	17.3	32	8.17	8.5		17.2	33	8.24	7.6	0.11	16.6	33	8.23	7.6	0.11	17.2	34	8.18	8.2	0.10	17.7	34
	2						8.15	8.5	0.10	17.0	32	8.25	7.5		16.5	33	8.20	7.4		17.0	33	8.13	8.2		17.6	34
	3						8.13	8.3		16.8	32	8.23	7.4		16.4	33	8.20	7.4		16.9	33	8.14	8.1		17.6	34
	4						8.20	8.2		16.5	33	8.19	7.4		16.2	33	8.13	7.4		16.6	34	7.98	8.0		17.3	34
	5						8.21	8.2		16.4	31	8.21	7.4		16.0	33	8.16	7.4		16.5	34	8.09	7.8		17.0	34
0.125	1	7.87	8.0	0.27	17.2	32	8.09	8.4		17.2	33	8.22	7.6	0.19	16.6	33	8.21	7.5	0.21	17.2	34	8.15	8.0	0.20	17.6	34
	2						8.02	8.4	0.22	17.0	33	8.24	7.5		16.5	33	8.21	7.4		17.1	33	8.16	8.0		17.6	34
	3						8.01	8.5		16.8	32	8.21	7.4		16.2	33	8.21	7.4		16.8	33	8.14	8.0		17.4	34
	4						8.03	8.3		16.5	33	8.25	7.4		16.0	33	8.25	7.4		16.5	34	8.21	8.0		17.0	34
	5						8.14	8.4		15.9	33	8.25	7.4		16.0	33	8.26	7.4		16.5	34	8.22	8.0		16.9	34
0.25	1	7.72	8.1	0.51	17.2	32	8.01	8.2		17.2	33	8.27	7.6	0.38	16.7	33	8.26	7.6	0.40	17.1	34	8.21	8.2	0.39	17.5	34
	2						8.01	8.2	0.70	17.0	33	8.26	7.6		16.5	33	8.27	7.6		17.0	34	8.20	8.0		17.5	34
	3						7.85	7.7		16.9	32	8.17	7.4		16.4	33	8.21	7.5		16.9	33	8.12	8.0		17.4	34
	4						8.02	7.8		16.5	33	8.23	7.4		16.0	33	8.22	7.4		16.6	34	8.15	7.8		17.0	34
	5						8.09	8.6		16.0	33	8.24	7.4		16.0	33	8.25	7.4		16.4	34	8.19	7.8		16.9	34
0.5	1	7.55	8.1	0.93	17.2	32	7.97	6.6		17.2	33	8.10	7.6	0.70	16.6	33	8.28	7.6	0.60	17.2	33	8.27	8.0	0.74	17.6	34
	2						7.84	7.7	0.40	17.0	32	8.20	7.4		16.5	33	8.23	7.5		17.0	33	8.19	8.0		17.6	34
	3						7.73	6.8		16.9	32	8.16	7.3		16.5	33	8.21	7.4		16.9	33	8.24	7.9		17.4	34
	4						7.78	7.6		16.6	33	8.13	7.2		16.3	33	8.21	7.4		16.6	34	8.18	7.8		17.2	34
	5						7.77	7.9		16.2	33	8.13	7.2		16.0	33	8.20	7.4		16.5	34	8.13	7.8		16.9	34
1	1	7.18	7.8	1.80	17.2	32	7.66	6.9		17.2	32	8.18	7.4	1.44	16.9	33	8.23	7.6	1.26	17.2	33	8.20	7.8	1.18	17.7	34
	2						7.81	7.1	1.50	17.0	32	8.23	7.3		16.6	33	8.28	7.4		17.1	33	8.26	7.8		17.7	34
	3						7.65	6.3		17.0	32	8.18	7.2		16.5	33	8.27	7.4		17.1	33	8.12	7.6		17.6	34
	4						7.60	5.9		16.7	33	8.14	7.2		16.2	33	8.23	7.3		16.7	32	8.17	7.6		17.3	34
	5						7.51	5.2		16.5	33	8.07	7.2		16.0	33	8.16	7.3		16.3	34	8.14	7.4		17.0	34
2.0	1	6.84	7.7	3.60	17.2	32	7.56	3.5		15.9	33	8.22	7.2	2.82	16.0	33	8.30	7.3	2.16	16.3	34	8.31	7.4	2.07	16.8	34
	2						7.47	2.0	3.70	15.7	33	8.09	7.2		16.0	34	—	—	—	—	—	—	—	—	—	—
	3						7.49	2.0		15.6	33	8.05	6.7		16.0	34	—	—	—	—	—	—	—	—	—	—
	4						7.38	0.6		15.8	33	8.14	6.7		16.0	34	—	—	—	—	—	—	—	—	—	—
	5						7.66	3.8		15.9	34	8.18	6.9		16.0	34	8.30	7.4		16.2	34	8.31	7.6		16.7	34
Min		6.84	7.7	0.03	17.1	32	7.38	0.6	0.03	15.6	31	8.05	6.7	0.02	16.0	33	8.13	7.2	0.03	16.2	32	7.98	7.4	0.03	16.7	34
Max		7.98	8.1	3.60	17.3	32	8.23	8.6	3.70	17.2	34	8.27	7.6	2.82	17.1	34	8.30	7.6	2.16	17.4	34	8.31	8.2	2.07	17.9	34

Note: — = All animals dead.

APPENDIX TABLE 6 (Cont'd)

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
0.06	**1**	7.84	8.1	0.24	17.6	32	8.15	8.1		17.2	33	8.26	7.2	0.16	16.6	33	8.28	7.6	0.20	17.1	34	8.27	8.2	0.18	17.6	34
	2						8.02	8.0	0.28	16.9	33	8.19	7.2		16.4	33	8.20	7.5		16.9	34	8.18	8.1		17.4	34
	3						8.18	8.0		16.5	33	8.24	7.2		16.0	33	8.26	7.4		16.7	34	8.24	8.1		17.2	34
	4						8.20	8.1		16.3	33	8.26	7.4		16.0	33	8.26	7.4		16.5	34	8.26	8.0		17.0	34
	5						8.20	8.0		16.2	34	8.25	7.4		16.0	33	8.15	7.5		16.5	34	8.27	8.0		17.0	34
0.125	**1**	7.79	8.1	0.47	17.7	32	8.12	8.1		17.2	33	8.25	7.5	0.27	16.5	34	8.28	7.4	0.32	17.0	34	8.27	8.2	0.28	17.4	34
	2						8.11	8.0	0.32	16.9	33	8.25	7.4		16.4	33	8.27	7.4		16.8	34	8.26	8.2		17.4	34
	3						8.05	8.0		16.6	33	8.21	7.4		16.2	33	8.26	7.4		16.6	34	8.12	8.0		17.2	34
	4						8.15	8.0		16.2	33	8.23	7.3		16.1	33	8.26	7.4		16.5	34	8.21	7.6		17.0	34
	5						8.17	8.1		16.2	33	8.27	7.4		16.0	34	8.27	7.6		16.5	34	8.26	7.6		16.9	34
0.25	**1**	7.66	8.0	0.84	17.6	32	7.95	7.8		17.1	33	8.24	7.4	0.54	16.4	33	8.26	7.6	0.51	16.9	34	8.25	8.0	0.47	17.4	34
	2						7.89	7.8	0.60	16.9	33	8.18	7.4		16.3	33	8.24	7.4		16.9	34	8.20	8.0		17.4	34
	3						7.93	7.8		16.6	33	8.20	7.2		16.2	33	8.24	7.4		16.6	34	8.21	7.9		17.2	34
	4						7.92	7.8		16.5	33	8.20	7.2		16.1	33	8.22	7.4		16.5	34	8.19	7.8		17.0	34
	5						8.01	7.8		16.2	33	8.20	7.2		16.0	34	8.25	7.4		16.5	34	8.23	7.8		16.9	34
0.5	**1**	7.43	7.9	1.60	17.6	32	7.89	7.8		17.1	33	8.25	7.4	1.10	16.2	33	8.27	7.5	1.05	16.8	34	8.26	8.0	0.98	17.2	34
	2						7.83	7.8	1.21	16.9	33	8.21	7.4		16.2	33	8.27	7.4		16.7	34	8.27	7.9		17.2	34
	3						7.79	7.4		16.7	33	8.20	7.2		16.1	33	8.27	7.4		16.6	34	8.23	7.8		17.2	34
	4						7.77	7.4		16.5	33	8.16	7.2		16.0	33	8.25	7.4		16.5	34	8.21	7.6		17.0	34
	5						7.94	7.8		16.2	33	8.24	7.2		16.0	34	8.30	7.4		16.5	34	8.28	7.6		16.9	34
1	**1**	7.10	7.8	3.20	17.6	32	7.64	5.8		16.9	33	8.25	7.3	2.21	16.0	34	—	—	—	—	—	—	—	—	—	—
	2						7.50	0.8	2.57	16.9	33	8.15	7.3		16.0	33	—	—	—	—	—	—	—	—	—	—
	3						7.62	5.2		16.6	33	8.20	7.2		16.0	33	8.24	7.4	2.05	16.5	34	8.28	7.8	2.01	17.0	34
	4						7.62	5.0		16.4	33	8.21	7.2		16.1	33	8.29	7.4		16.5	34	8.31	7.6		16.9	34
	5						7.67	4.8		16.2	33	8.17	7.2		16.0	34	8.25	7.3		16.5	34	8.22	7.6		16.9	34
2.0	**1**	6.82	7.2	6.10	17.9	32	7.45	0.8		17.0	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.49	0.4	5.28	16.7	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.40	0.6		16.5	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.57	1.8		16.3	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.47	0.6		16.2	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.82	7.2	0.24	17.6	32	7.40	0.4	0.28	16.2	33	8.15	7.2	0.16	16.0	33	8.15	7.3	0.20	16.5	34	8.12	7.6	0.18	16.9	34
Max		7.84	8.1	6.10	17.9	32	8.20	8.1	5.28	17.2	34	8.27	7.5	2.21	16.6	34	8.30	7.6	2.05	17.1	34	8.31	8.2	2.01	17.6	34

Note: — = All animals dead.

APPENDIX TABLE 7

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	9	9	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	98.0
0.06	1	10	10	9	9	9	90	
	2	10	10	9	10	10	100	
	3	10	10	10	9	9	90	
	4	10	9	9	8	8	80	
	5	10	9	9	9	9	90	90.0
0.125	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.25	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.5	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	9	9	90	98.0
1	1	10	10	10	10	9	90	
	2	10	10	10	10	6	60	
	3	10	10	10	10	7	70	
	4	10	10	10	10	6	60	
	5	10	10	8	6	5	50	66.0
2	1	10	*	3	3	1	10	
	2	10	*	0	—	—	0	
	3	10	*	0	—	—	0	
	4	10	*	0	—	—	0	
	5	10	*	2	2	1	10	4.0

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 7 (Cont'd)

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.06	1	10	10	10	10	10	100	
	2	10	10	7	6	5	50	
	3	10	10	10	10	10	100	
	4	10	10	7	7	6	60	
	5	10	10	9	9	9	90	80.0
0.125	1	10	10	10	10	10	100	
	2	10	10	9	9	8	80	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	94.0
0.25	1	10	10	10	10	9	90	
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	9	9	90	
	5	10	10	8	8	7	70	86.0
0.5	1	10	10	9	9	9	90	
	2	10	10	10	9	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	9	9	8	80	88.0
1	1	10	*	0	—	—	0	
	2	10	*	0	—	—	0	
	3	10	*	2	2	3	30	
	4	10	*	2	2	2	20	
	5	10	*	2	2	2	20	14.0
2	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 8

Mysidopsis bahia
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration		Day 0				Day 1				Day 2				Day 3				Day 4			
(mg/L)	Rep	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
0.7	1	8.06	8.2	15.9	33	8.16	7.2	17.4	33	8.16	7.2	17.4	33	8.03	7.4	17.6	33	7.88	6.8	18.2	33
	2					8.19	7.1	17.2	33	8.16	7.2	17.3	33	8.07	7.4	17.6	33	7.91	6.7	18.2	33
	3					8.20	7.1	17.3	33	8.16	7.1	17.3	33	8.06	7.2	17.6	33	7.88	6.6	18.2	33
1.25	1	8.07	8.1	15.9	32	8.19	7.0	17.2	33	8.17	7.0	17.3	33	8.08	7.2	17.6	33	7.93	6.5	18.2	33
	2					8.19	7.0	17.0	33	8.16	7.0	17.2	33	8.07	7.2	17.6	33	7.93	6.6	18.0	33
	3					8.19	7.0	17.1	33	8.15	7.1	17.2	33	8.07	7.2	17.5	33	7.93	6.6	18.0	33
2.5	1	8.07	8.1	15.8	32	8.16	6.9	17.2	33	8.13	7.0	17.3	33	8.05	7.2	17.6	33	7.93	6.7	18.2	33
	2					8.15	6.5	17.0	33	8.12	7.0	17.0	33	8.05	7.2	17.5	33	7.96	6.6	18.0	33
	3					8.14	6.4	17.0	33	8.12	7.0	17.1	33	8.03	7.2	17.6	33	7.89	6.7	18.0	33
5	1	8.08	8.1	15.9	32	8.11	6.4	17.2	33	8.08	7.0	17.4	33	8.02	7.2	17.6	33	7.90	6.5	18.3	33
	2					8.11	6.0	17.0	33	8.08	6.8	17.3	33	8.01	7.0	17.6	33	7.91	6.5	18.1	33
	3					8.10	5.8	17.0	33	8.09	6.8	17.2	33	8.00	7.0	17.6	33	7.89	6.4	18.2	33
10	1	8.08	8.0	15.8	32	8.05	5.8	17.3	33	8.01	6.4	17.5	33	7.98	7.0	17.9	33	7.89	6.4	18.6	33
	2					8.07	5.8	17.1	33	7.99	6.4	17.3	33	7.98	7.0	17.8	33	7.89	6.4	18.3	33
	3					8.08	5.1	17.2	33	7.98	6.4	17.3	33	7.98	7.0	17.6	33	7.87	6.4	18.3	33
20	1	8.09	8.0	15.8	32	8.05	4.8	17.5	33	7.80	4.5	17.7	33	—	—	—	—	—	—	—	—
	2					8.06	4.7	17.3	33	7.77	4.4	17.6	33	7.83	7.1	18.0	33	7.85	6.4	18.7	33
	3					8.05	4.7	17.2	33	7.78	4.4	17.4	33	7.81	6.4	17.8	33	7.92	6.7	18.6	34
40	1	8.09	8.1	15.7	32	8.12	6.0	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
	2					8.17	6.2	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
	3					8.17	6.2	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
Min		8.06	8.0	15.7	32	8.05	4.7	17.0	33.0	7.77	4.4	17.0	33.0	7.81	6.4	17.5	33.0	7.85	6.4	18.0	33.0
Max		8.09	8.2	15.9	33	8.20	7.2	17.8	33.0	8.17	7.2	17.7	33.0	8.08	7.4	18.0	33.0	7.96	6.8	18.7	34.0

Note: — = All animals dead.

APPENDIX TABLE 9

Mysidopsis bahia
SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.7	**1**	10	10	9	8	8	80	
	2	10	10	10	10	10	100	
	3	10	10	9	9	9	90	90.0
1.25	**1**	10	10	9	9	9	90	
	2	10	10	9	9	9	90	
	3	10	10	10	10	9	90	90.0
2.5	**1**	10	10	8	8	8	80	
	2	10	10	7	7	7	70	
	3	10	9	8	8	7	70	73.3
5	**1**	10	10	10	10	10	100	
	2	10	10	7	7	6	60	
	3	10	9	9	9	9	90	83.3
10	**1**	10	10	9	8	8	80	
	2	10	8	7	7	7	70	
	3	10	8	7	6	6	60	70.0
20	**1**	10	2	0	—	—	0	
	2	10	2	2	2	2	20	
	3	10	1	1	1	1	10	10.0
40	**1**	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	0.0

Note: — = All animals dead.

APPENDIX TABLE 10

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
Study Dates: 10/26-10/30/94
HSW-1

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	8.6	<0.01	16.5	32	8.05	8.2	0.08	16.8	32	8.03	8.8	0.08	14.6	33	7.94	6.8	0.08	15.4	33	7.95	8.2	0.09	15.7	33
	2						7.92	8.3	0.08	16.9	32	7.82	8.8	0.09	14.7	33	7.78	7.0	0.09	15.5	33	7.81	8.2	0.14	15.7	33
	3						7.91	7.8	0.07	16.9	32	7.84	9.0	0.09	14.6	33	7.79	6.8	0.07	15.5	33	7.81	7.2	0.19	15.7	33
	4						8.04	8.1	0.07	16.8	32	7.99	8.7	0.08	14.5	33	8.00	6.6	0.07	15.4	33	7.99	8.1	0.18	15.6	33
	5						8.00	8.2	0.07	16.8	32	7.99	8.8	0.09	14.6	33	7.94	6.6	0.08	15.4	33	7.97	8.1	0.17	15.6	33
0.06	1	7.95	8.6	0.16	16.4	32	7.90	8.1	0.14	16.7	32	8.00	9.0	0.17	14.6	33	7.99	7.2	0.16	15.4	33	8.00	8.1	0.29	15.7	33
	2						7.89	8.0	0.14	16.6	32	8.01	9.0	0.17	14.5	33	8.00	7.2	0.18	15.5	33	8.03	8.1	0.26	15.6	34
	3						7.95	8.0	0.14	16.5	32	8.04	9.0	0.17	14.5	33	8.04	7.0	0.14	15.4	33	8.06	8.3	0.29	15.5	34
	4						7.83	7.6	0.15	16.3	32	8.02	9.0	0.18	14.2	33	7.94	7.2	0.18	15.3	33	7.95	8.2	0.30	15.2	34
	5						7.82	7.8	0.15	16.2	32	7.97	8.9	0.18	14.2	33	7.93	7.2	0.17	15.4	33	7.96	7.9	0.31	15.0	33
0.125	1	7.93	8.6	0.23	16.4	32	7.61	5.1	0.21	16.3	32	7.99	8.9	0.21	14.2	33	7.98	7.4	0.20	15.4	33	8.01	8.1	0.35	15.3	34
	2						7.59	5.0	0.22	16.2	32	7.99	9.0	0.24	14.2	33	7.95	7.2	0.24	15.2	33	8.01	8.1	0.40	15.2	34
	3						7.76	7.2	0.22	16.0	32	8.01	9.1	0.23	14.2	33	7.97	7.2	0.20	15.4	33	8.03	8.2	0.48	15.4	34
	4						7.64	5.6	0.19	16.2	32	8.01	9.1	0.23	14.3	33	7.97	7.0	0.19	15.2	33	8.00	8.1	0.53	15.3	34
	5						7.86	7.3	0.19	16.2	32	8.03	9.1	0.23	14.2	33	8.04	7.0	0.21	15.3	33	8.08	8.0	0.51	15.2	34
0.25	1	7.83	8.6	0.47	16.5	32	7.58	4.6	0.35	16.0	32	7.94	9.0	0.37	13.9	34	7.90	7.2	0.34	15.3	33	7.97	8.1	0.53	14.5	36
	2						7.65	4.7	0.36	16.0	32	8.04	8.8	0.37	14.0	33	8.01	7.3	0.33	15.3	33	8.10	8.0	0.62	14.7	35
	3						7.62	4.6	0.35	16.0	32	8.07	8.9	0.36	14.3	33	8.03	7.3	0.37	15.4	33	8.10	8.2	0.57	14.9	34
	4						7.67	4.7	0.34	15.9	32	8.03	9.0	0.36	14.4	33	7.92	7.3	0.36	15.4	33	8.03	8.2	0.66	15.1	34
	5						7.67	4.8	0.34	16.0	32	8.08	9.1	0.36	14.3	33	8.05	7.2	0.37	15.3	33	8.11	8.3	0.61	14.9	35
0.5	1	7.63	8.5	0.92	16.4	32	7.50	1.2	0.74	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.50	0.9	0.67	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.52	0.8	0.76	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.51	1.3	0.75	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.57	1.0	0.66	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	1	7.33	8.5	1.98	16.4	31	7.45	0.8	1.58	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.46	0.9	1.62	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.47	0.6	1.59	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.48	0.8	1.54	16.4	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.46	0.8	1.63	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	1	6.99	8.1	3.95	16.5	31	7.41	0.6	3.18	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.40	0.4	3.20	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.48	0.6	3.12	16.0	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.41	0.8	3.15	16.1	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.45	0.8	3.19	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.99	8.1	<0.10	16.4	31	7.40	0.4	0.07	15.9	32	7.82	8.7	0.08	13.9	33	7.78	6.6	<0.10	15.2	33	7.81	7.2	0.09	14.5	33
Max		8.02	8.6	3.95	16.5	32	8.05	8.3	3.20	16.9	32	8.08	9.1	0.37	14.7	34	8.05	7.4	0.37	15.5	33	8.11	8.3	0.66	15.7	36

Note: — = All animals dead.

APPENDIX TABLE 10 (Cont'd)

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
Study Dates: 10/26-10/30/94
HSW-2

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
0.06	1	8.00	8.5	0.19	16.5	32	7.76	7.0	0.20	16.5	32	8.03	9.2	0.17	14.8	32	8.07	7.4	0.17	15.5	33	8.09	8.2	0.17	15.5	33
	2						7.84	7.2	0.17	16.4	32	8.03	9.1	0.17	14.4	33	8.04	7.2	0.16	15.4	33	8.08	8.3	0.20	15.5	33
	3						7.84	7.2	0.18	16.3	32	8.02	9.1	0.18	14.2	33	8.05	7.2	0.18	15.5	33	8.08	8.3	0.21	15.3	34
	4						7.75	6.2	0.17	16.4	32	8.00	9.0	0.18	14.2	33	8.01	7.0	0.17	15.5	33	8.06	8.2	0.19	15.2	34
	5						7.79	6.6	0.18	15.9	32	8.04	8.9	0.18	14.5	33	8.05	7.1	0.19	15.4	33	8.10	8.2	0.23	14.4	36
0.125	1	7.94	8.6	0.30	16.5	32	7.70	6.4	0.27	16.2	32	7.99	8.9	0.26	14.2	33	8.02	7.5	0.21	15.4	33	8.06	8.3	0.31	15.3	34
	2						7.81	6.2	0.27	16.3	32	8.03	9.1	0.27	14.3	33	8.04	7.3	0.25	15.4	33	8.09	8.1	0.34	15.3	34
	3						7.81	6.0	0.27	16.4	32	8.04	9.2	0.26	14.3	33	8.05	7.2	0.25	15.5	33	8.10	8.3	0.29	15.3	34
	4						7.58	6.1	0.29	15.9	32	8.04	9.2	0.26	13.8	33	8.06	7.2	0.27	15.3	33	8.11	8.3	0.31	14.8	35
	5						7.76	6.2	0.29	15.9	32	8.06	9.2	0.25	13.8	33	8.07	7.2	0.27	15.3	33	8.13	8.3	0.34	14.8	34
0.25	1	7.79	8.6	0.62	16.4	32	7.70	4.2	0.57	15.9	32	7.94	9.2	0.47	13.9	33	8.00	7.4	0.44	15.2	33	8.05	8.3	0.47	14.9	34
	2						7.70	4.5	0.58	15.9	32	7.91	8.9	0.47	13.8	33	7.96	7.2	0.41	15.3	33	8.02	8.2	0.49	14.9	34
	3						7.64	4.6	0.55	15.9	32	7.98	8.8	0.47	13.8	33	7.99	7.2	0.41	15.3	33	8.07	8.0	0.41	14.8	34
	4						7.61	4.6	0.53	16.1	32	7.89	8.8	0.46	14.0	33	7.92	7.3	0.40	15.3	33	8.00	8.1	0.47	15.2	34
	5						7.59	4.6	0.52	16.2	32	7.92	8.8	0.47	14.2	33	7.91	7.2	0.43	15.3	33	7.98	7.9	0.49	15.2	34
0.5	1	7.54	8.7	1.24	16.5	32	7.57	1.6	1.07	16.2	32	7.97	8.7	0.87	14.0	33	8.04	7.0	0.79	15.4	33	8.08	8.2	0.74	14.9	34
	2						7.49	1.8	1.16	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.54	1.8	1.09	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.56	1.8	1.08	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.57	1.9	1.03	16.3	32	8.05	8.8	0.86	14.2	33	8.09	7.0	0.83	15.4	33	8.15	8.2	0.69	15.0	35
1	1	7.23	8.6	2.41	16.5	32	7.61	0.9	2.10	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.62	0.9	2.24	16.3	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.54	1.0	2.22	16.4	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.54	0.8	2.31	15.8	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.51	0.8	2.31	15.7	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2.0	1	6.86	8.3	5.15	16.5	31	7.80	0.6	4.88	15.8	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.56	0.6	4.47	15.9	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.60	0.8	4.65	15.9	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.60	0.8	4.40	16.0	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.56	0.6	4.32	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.86	8.3	0.19	16.4	31	7.49	0.6	0.17	15.7	32	7.89	8.7	0.17	13.8	32	7.91	7.0	<0.10	15.2	33	7.98	7.9	0.19	14.4	33
Max		8.00	8.7	5.15	16.5	32	7.84	7.2	4.88	16.5	32	8.06	9.2	0.87	14.8	33	8.09	7.5	0.83	15.5	33	8.15	8.3	0.74	15.5	36

Note: — = All animals dead.

APPENDIX TABLE 11

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.06	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.125	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.25	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.5	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
1	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
2	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Note: — = All animals dead.

APPENDIX TABLE 11 (Cont'd)

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.06	**1**	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.125	**1**	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	9	10	10	100	100.0
0.25	**1**	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.5	**1**	10	4	2	2	2	20	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	2	2	2	2	20	8.0
1	**1**	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
2	**1**	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Note: — = All animals dead.

APPENDIX TABLE 12

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration		Day 0				Day 1			
(mg/L)	Rep	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	**1**	7.93	9.4	15.4	31	7.75	5.0	NT	NT
	2					7.73	4.8	NT	NT
	3					7.69	4.8	NT	NT
1.6	**1**	7.94	9.4	15.2	31	7.62	4.0	NT	NT
	2					7.68	4.4	NT	NT
	3					7.70	4.4	NT	NT
3.1	**1**	7.95	9.4	15.2	31	7.59	4.1	NT	NT
	2					7.61	4.3	NT	NT
	3					7.64	4.4	NT	NT
6.25	**1**	7.95	9.4	15.2	31	7.42	2.1	NT	NT
	2					7.72	2.1	NT	NT
	3					7.75	2.2	NT	NT
12.5	**1**	7.96	9.4	15.2	31	7.42	2.0	NT	NT
	2					7.59	2.1	NT	NT
	3					7.56	2.1	NT	NT
25	**1**	7.96	9.4	15.2	31	7.40	2.0	NT	NT
	2					7.43	2.0	NT	NT
	3					7.48	2.0	NT	NT
Min		7.93	9.4	15.2	31	7.40	2.0		
Max		7.96	9.4	15.4	31	7.75	5.0		

Note: NT = Not taken.

APPENDIX TABLE 13

Citharichthys stigmaeus
SURVIVAL DATA
FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	% Survival	Average % Survival
Control	1	6	6	100	
	2	6	6	100	
	3	6	6	100	100.0
1.6	1	6	6	100	
	2	6	6	100	
	3	6	6	100	100.0
3.1	1	6	5	83	
	2	6	5	83	
	3	6	5	83	83.3
6.25	1	6	0	0	
	2	6	0	0	
	3	6	0	0	0.0
12.5	1	6	0	0	
	2	6	0	0	
	3	6	0	0	0.0
25	1	6	0	0	
	2	6	0	0	
	3	6	0	0	0.0

ATTACHMENT 2

Standard Operating Procedures

High Strength Waste Sampling

for Bioassay Toxicity Tests

Standard Operating Procedures
High Strength Waste Sampling
for Bioassay Toxicity Tests

Introduction

Starkist Samoa, Inc. and VCS Samoa Packing are each required under their Ocean Disposal Dumping Permits to conduct definitive acute bioassays on their high strength waste (HSW) streams that are barged to sea for disposal at the permitted dump site. The following gives detailed procedures for collecting, preparing, and shipping samples for these analyses.

Each cannery is required to collect a composite sample of high strength waste while the waste is being transferred from the storage tanks to the barge. Currently a one gallon composite is required for the bioassay tests. The procedures described below are applicable to sampling at each of the canneries.

List of Equipment/Supplies

The following supplies will be required for collecting composite high strength waste samples and preparing them for delivery to the laboratories:

- Three (3) 1/2 to 1 gallon sampling containers
- One 1-gallon cubitainer or other appropriate container (container should be heavy-duty plastic with secure cap, do not ship samples in glass containers)
- Permanent marker for marking sample containers
- Cooler with ice (or refrigerator space) for storing sample
- Cooler for shipping samples (note: Cooler should be sized to hold sample(s) with sufficient room for ice.)
- Cubed ice (enough ice to fill airspace in cooler)
- Chain of Custody Forms (supplied by CH2M HILL or by laboratory conducting the analysis)

Sampling

The following describes the general sampling procedures:

1) **Collect "Grab" Samples.** Sampling should take place the day of or evening before the samples are shipped to the lab. Collect three 1/2 to 1-gallon grab samples from existing sampling ports in the storage tank transfer lines at the time waste is being transferred from the storage tanks to the barge. The samples should be collected at 10 minute intervals. Record the time each grab was taken. Store all samples in coolers on ice or in a

refrigerator at a temperature of approximately 4°C. Do **NOT** store samples in a freezer or using a method that would otherwise freeze the samples.

2) **Composite Samples.** Using a permanent marker, label the 1-gallon cubitainer with the following information:

- Facility samples were collected from
- Date
- Time each grab sample was collected

Combine the three grab samples by measuring 1/3 gallon of each into the 1-gallon cubitainer. Seal the sample container by placing plastic inside the cap and taping the cap down.

3) **Complete Chain of Custody Form.** One chain-of-custody form is required for each cooler in which samples are shipped. An example of a completed chain-of-custody form is included as Attachment A, along with a blank copy. Fill out the chain-of-custody form in triplicate or copy keeping one copy and sending two with the samples to the laboratory.

Shipping

The samples should be shipped the fastest way possible to:

Dr. Kurt Kline
Advanced Biological Testing, Inc.
3150 Paradise Drive, Building 50
Tiburon, CA 94920

Phone: (415) 435-7878; Fax: (415) 435-7882

The samples from each cannery can be shipped in separate coolers or in the same cooler. Place the composite sample into the cooler in which sample(s) is to be shipped. Ice, or an equivalent means such as chemical cold packs, should be used to fill in the empty space in the cooler and keep the sample(s) cold during shipping. Do not use dry ice to ship the sample. If cubed ice is used, precautions should be taken to prevent the melted ice from leaking out of the cooler during shipping. These include taping any drain plugs in the cooler shut with duct tape or strapping tape, and "double-bagging" the ice cubes in zip-lock bags, i.e. sealing the ice cubes in one bag, then sealing the bag containing ice in a second bag. As much air as possible should be removed from the bags prior to sealing. (Too much air inside the bags will expand during flight and pop the bag open).

The chain-of-custody form should signed, placed in a zip-lock bag, and taped with duct tape to the inside of the cooler lid. The cooler should be taped securely with strapping tape or other strong packaging tape to prevent it from opening during shipping.

Attachment A
Example Chain-of-Custody Form

CH2M HILL

QUALITY ANALYTICAL LABORATORIES

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M HILL Project #: OPE3702.DS.BT

Purchase Order #:

Project Name: OCEAN DUMPING PERMIT HIGH STRENGTH WASTE BIOASSAY

Company Name/~~CH2M HILL Office~~: CH2M HILL / SFO

Project Manager & Phone #: Mr. [ ] Ms. [ ] Dr. [X] STEVE COSTA ~~510~~ 510 251-2888 x2251

Report Copy to: SAME

Requested Completion Date: A.S.A.P

Sampling Requirements: SDWA [ ] NPDES [ ] RCRA [ ] OTHER OCN

Sample Disposal: Dispose [X] Return [ ]

LAB TEST CODES

ANALYSES REQUESTED

SHADED AREA -- FOR LAB USE ONLY

Lab 1 #: Lab 2 #:

Quote #: Kit Request #:

Project #:

No. of Samples: Page: of:

COC Rev: Login: LIMS Ver: Ack Gen:

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	CLIENT SAMPLE ID (9 CHARACTERS)	# OF CONTAINERS	BIOASSAY GROUP 1	BIOASSAY GROUP 2	BIOASSAY GROUP 3	REMARKS	LAB 1 ID	LAB 2 ID
10/18	1000	X		X		VCS SAMOA1	1	X	X	X	1 - 1gal cubitainer ON ICE		

SAMPLE

Do Not Use

Sampled By & Title: Barry Fowler (Please sign and print name) BARRY FOWLER

Date/Time: 10/18 1004

Relinquished By (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Relinquished By (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Relinquished By (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Shipped Via: UPS BUS Fed-Ex Hand Other

Shipping #:

Work Authorized By (Please sign and print name)

Remarks: SAMPLE IS COMPOSITE OF 3 GRAB SAMPLES TAKEN AT 10 MINUTE INTERVALS

HAZWRAP/NESSA: Y N

QC Level: 1 2 3 Other:

COC Rec | ICE

Ana Req | TEMP

Cust Seal | Ph

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: ORIGINAL - LAB, Yellow - LAB, Pink - Client

REV 11/92 FORM 340

CHAIN OF CUSTODY INSTRUCTIONS

CH2M HILL Project #:	CH2M HILL project number to be charged for work.
Purchase Order #:	Purchase order to be charged for work (OTC clients).
Project Name:	Name of project which the samples support.
Company Name/CH2M HILL Office:	Name of the company or CH2M HILL office requesting the work. Correspondence will be sent to the company address or CH2M HILL office.
Project Manager & Phone #:	Name and phone number of person who receives the laboratory report and can be contacted if questions arise.
Report Copy To:	Name and location of person to receive copy of laboratory report.
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   CH2M HILL Quality Analytical Laboratories make no warranty, express or implied, and under no circumstances will be liable for any claims or damages except those resulting solely from their own or their employees' negligence. To the maximum extent permitted by law, our liability for damages will not exceed the compensation received by CH2M HILL Quality Analytical Laboratories under the project Agreement.

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7. **Interpretation**
   The limitations of liability and indemnities will apply whether CH2M HILL's liability arises under breach of contract or warranty; tort, including negligence (but not sole negligence); strict liability; statutory liability; or any other causes of action; and shall apply to CH2M HILL's officers, employees, and subcontractors. The professional services agreement will take precedence in the event there is a conflict with the agreement and chain-of-custody document.

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CH2M HILL

QUALITY ANALYTICAL LABORATORIES

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M HILL Project # OPE3070 2.DS.BT

Purchase Order #

Project Name: OCEAN DUMPING PERMIT HIGH STRENGTH WASTE BIOASSAY

Company Name/CH2M HILL Office: CH2M HILL / SFO

Project Manager & Phone #: Mr. [ ] Ms. [ ] Dr. [X] STEVE COSTA 510 251-2888 X2251

Report Copy to: SAME

Requested Completion Date: A.S.A.P.

Sampling Requirements: SDWA [ ] NPDES [ ] RCRA [ ] OTHER OCN

Sample Disposal: Dispose [X] Return [ ]

LAB TEST CODES

SHADED AREA -- FOR LAB USE ONLY

Lab 1 # | Lab 2 #

Quote # | Kit Request #

ANALYSES REQUESTED

Project #

No. of Samples | Page | of

COC Rev | Login | LIMS Ver | Ack Gen

REMARKS | LAB 1 ID | LAB 2 ID

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	CLIENT SAMPLE ID (9 CHARACTERS)	# OF CONTAINERS	BIOASSAY GROUP 1	BIOASSAY GROUP 2	BIOASSAY GROUP 3	REMARKS	LAB 1 ID	LAB 2 ID
10/18	1000	X		X		STARKIST	1	X	X	X	1 - 1gal cub. tainer ON ICE		

SAMPLE DO NOT USE

Sampled By & Title (Please sign and print name): Cliff Johnson CLIFF JOHNSON — Date/Time: 10/18 1000

Relinquished By (Please sign and print name) — Date/Time

HAZWRAP/NESSA: Y N

Received By (Please sign and print name) — Date/Time

Relinquished By (Please sign and print name) — Date/Time

QC Level: 1 2 3 Other:

COC Rec | ICE

Received By (Please sign and print name) — Date/Time

Relinquished By (Please sign and print name) — Date/Time

Ana Req | TEMP

Cust Seal | Ph

Received By (Please sign and print name) — Date/Time

Shipped Via: UPS BUS Fed-Ex Hand Other DHL

Shipping #

Work Authorized By (Please sign and print name)

Remarks: SAMPLE IS COMPOSITE OF 3 GRAB SAMPLES TAKEN AT 10 MINUTE INTERVALS

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: ORIGINAL - LAB, Yellow - LAB, Pink - Client

REV 11/92 FORM 340

CHAIN OF CUSTODY INSTRUCTIONS

CH2M HILL Project #:	CH2M HILL project number to be charged for work.
Purchase Order #:	Purchase order to be charged for work (OTC clients).
Project Name:	Name of project which the samples support.
Company Name/CH2M HILL Office:	Name of the company or CH2M HILL office requesting the work. Correspondence will be sent to the company address or CH2M HILL office.
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CH2M HILL

QUALITY ANALYTICAL LABORATORIES

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M HILL Project #

Purchase Order #

Project Name

Company Name/CH2M HILL Office

Project Manager & Phone #
Mr. [ ]
Ms. [ ]
Dr. [ ]

Report Copy to:

Requested Completion Date:

Sampling Requirements: SDWA ☐ NPDES ☐ RCRA ☐ OTHER ____

Sample Disposal: Dispose ☐ Return ☐

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	CLIENT SAMPLE ID (9 CHARACTERS)	# OF CONTAINERS

LAB TEST CODES

ANALYSES REQUESTED

SHADED AREA -- FOR LAB USE ONLY

Lab 1 #

Lab 2 #

Quote #

Kit Request #

Project #

No. of Samples

Page

of

COC Rev

Login

LIMS Ver

Ack Gen

REMARKS

LAB 1 ID

LAB 2 ID

Sampled By & Title (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Received By (Please sign and print name) Date/Time

Work Authorized By (Please sign and print name)

Relinquished By (Please sign and print name) Date/Time

Relinquished By (Please sign and print name) Date/Time

Relinquished By (Please sign and print name) Date/Time

Shipped Via
UPS BUS Fed-Ex Hand Other____

Shipping #

Remarks

HAZWRAP/NESSA: Y N

QC Level: 1 2 3 Other: ____

COC Rec | ICE

Ana Req | TEMP

Cust Seal | Ph

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: ORIGINAL - LAB, Yellow - LAB, Pink - Client

REV 11/92 FORM 340

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ATTACHMENT 3

1 July 1994 Memo to EPA

Recommending Changes to the High Strength Waste

Bioassay Testing Protocols

MEMORANDUM CH2MHILL

TO: Pat Young/USEPA

COPIES: Amy Wagner/USEPA (w/ attachments)
Kurt Kline/ABT (w/o attachments)

FROM: Steve Costa/CH2M HILL/SFO
Don Kingery/CH2M HILL/SFO

DATE: July 1, 1994

SUBJECT: Bioassay Testing of Starkist Samoa, Inc. and VCS Samoa Packing High Strength Waste

PROJECT: OPE030702.EL.R2

High strength waste (HSW) bioassays are required by Special Condition 3.3.5 of Starkist Samoa's and VCS Samoa Packing's ocean dumping permits. The results of the tests are presented in the attached: "*Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa*" prepared by Advanced Biological Testing Inc., Tiburon, California.

Acute effluent bioassays were conducted on *Mysidopsis bahia* (mysid shrimp) juveniles, *Mytilus edulis* (blue mussel) larvae, *Strongylocentrotus purpuratus* (purple sea urchin) larvae, and *Citharichthys stigmaeus* (speckled sanddab) juveniles using HSW collected separately from the Starkist Samoa and VCS Samoa Packing canneries in Pago Pago Harbor, American Samoa. The results of these bioassays are summarized in the table below.

Based on the results of the bioassays, we recommend the following changes to the HSW bioassay protocol:

Reduce the upper end of the HSW concentration series for all bioassays to a maximum of 3.0%. The results of the bioassay tests give a better understanding of the test concentrations needed. No additional information is required at concentrations greater than 3.0%. Reducing the maximum concentrations will reduce the amount of HSW that needs to be sampled and shipped. We recommend a series of concentrations for the bioassays of 3.0%, 1.5%, 0.8%, 0.2%, 0.1%, and 0.05%.

Continue running bioassays with *Mytilus edulis* while monitoring the effects of aeration on organism mortality but drop the use of *Strongylocentrotus purpuratus* larvae as test organisms for the HSW. This recommendation is made for the following reasons:

- Special Condition 3.3.5 of the permits requires only three organisms be tested; one organism each out of three specified groups. *Mysidopsis bahia* and

Citharichthys stigmaeus satisfy the requirements for Groups 2 and 3. Group 1 contains larval stages of both bivalves and echinoderms and running just *Mytilus edulis* should satisfy this requirement.

- Because of the high oxygen demand of the effluent, all test containers required aeration throughout the tests to maintain adequate oxygen concentrations for the test organisms. Aerating the chambers using *Mytilus edulis* and *Strongylocentrotus purpuratus* larvae as bioassay test organisms gives problematic results. Aeration is standard protocol for bioassays on fish and invertebrates when oxygen levels fall below 40% of saturation, but is not standard protocol for bioassays on larval bivalves and echinoderms. The effects of aerating the water on the survival of these organisms is not known. Because the *Mytilus edulis* bioassays are only run for two days (vs. four for the *Strongylocentrotus purpuratus*) the organisms are exposed for half the time and the effects of aeration may be reduced.

- The mortality of the control group was substantial for the echinoderms and is unacceptable according to protocol. The cause of the high mortality in the control is not known at this time.

Please review the above recommendations. We suggest Amy Wagner contact Kurt Kline, Advanced Biological Testing Inc., directly at (415)435-7878 to discuss any comments you have on the bioassay protocols.

Summary of High Strength Waste Bioassay Results.

Test Organism	Starkist Samoa		VCS Samoa Packing	
	LC_{50}	NOEC/IC_{50} [1]	LC_{50}	NOEC/IC_{50} [1]
Citharichthys stigmaeus (sanddab)	0.27%	0.2%	0.59%	0.4%
Mysidopsis bahia (mysid shrimp)	0.12%	0.05%	0.59%	0.05%
Mytilus edulis (blue mussel)	> 1.2%	< 0.08%	> 1.2%	< 0.08
Strongylocentrotus purpuratus [2] (urchin)	> 1.2%	< 0.08%	> 1.2%	0.1%

[1] NOEC reported for the juvenile sanddabs and mysid shrimp, IC_{50} reported for the mussel and urchin larvae.

[2] Control survival of 64.4% is unacceptable according to protocol.

ATTACHMENT 4

29 August 1994 Response From EPA

Containing Acceptable Changes to the

High Strength Waste Bioassay Testing Protocols

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

August 29, 1994

Steven L. Costa
Project Manager
CH2M Hill
P.O. Box 12681
Oakland, CA 94604-2681

Re: Comments on Bioassay Testing of Ocean Disposed High-Strength Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

Dear Steve:

We have reviewed the report of June 29, 1994 for the first of three rounds of bioassays of high-strength waste, as required by the canneries' ocean disposal permits. The report is based on two sampling events: the first was collected on February 16, 1994; and, a second sample was required and tested in March 1994, due to test failure of the echinoderms in the first sample. Your proposed changes to the study methods, as outlined in your memo of July 1, 1994, are acceptable. Enclosed is a memo from Amy Wagner of EPA's Laboratory Support Section, detailing the acceptable changes. Please call Amy at (510) 412-2329 if you have any questions on her comments.

We note that the second and third rounds of testing were scheduled for May and August 1994, and we would like to know if these tests were conducted as scheduled and, if not, the rescheduled dates, and when we can anticipate the reports on these bioassays. Please relay this information to Pat Young, American Samoa Program Manager, or if you have any questions, call her at (415) 744-1594.

Sincerely,

for M. J. Lee
Norman L. Lovelace, Chief
Office of Pacific Island and Native American Programs (E-4)

Enclosure

cc: Jim Cox, Van Camp Seafood Company
Norman Wei, StarKist Seafood Company
Tony Tausaga, American Samoa EPA
Sheila Wiegman, American Samoa EPA
Allan Ota, W-3-3
Amy Wagner, P-3-1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX LABORATORY
1337 S. 46TH STREET BLDG 201
RICHMOND, CA 94804-4698

AUG 2 9 1994

MEMORANDUM

SUBJECT: Review of Bioassay Testing of Starkist, Samoa, Inc. and VCS Samoa Packing High Strength

FROM: Amy Wagner
Laboratory Section (P-3-1)

THRU: Brenda Bettencourt, Chief
Laboratory Section (P-3-1)

TO: Pat Young
OPINAP (E-4)

Allan Ota
Wetlands and Sediment Management Section (W-3-3)

At your request, I have reviewed "Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa." The following recommendations are based on the results of the first round of testing.

1. p. 11. The salinity of the *Mysidopsis bahia* tests were 25 ppt, presumably based on the salinity of the shipping water. An effort should be made to find a supplier that raises mysids in a salinity closer to that of the discharge site, between 30-35 ppt.

2. Appendix, p. 1. It is recommended that the water quality measurements pH, dissolved oxygen, and initial salinity be measured for all samples upon receipt.

3. Appendix, Table 10. The salinities of 26-28 ppt most likely caused the high mortality in controls with the sea urchin toxicity test. If necessary, brine adjustments should be used to increase the salinity of test samples to the test method requirements of 30 $\pm$ 2 ppt.

4. To reduce salinity elevation throughout the tests, an attempt should be made to cover test containers to reduce evaporation.

Based on the results of these tests, the following changes in the bioassay methods recommended by CH2M Hill in the cover memo are acceptable.

1. The series of the concentrations for toxicity tests can be reduced to 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, and 0.0625% instead of the suggested series.

2. *Mytilus edulis* can be used instead of *Strongylocentrotus purpuratus* as the third test organism. The oyster *Crassostrea virginica* may be substituted for the mussel test during the months when mussels cannot be spawned.

3. Aeration should be provided in the mussel test containers due to high biological oxygen demand of the effluent. In addition to a control with aeration, a control without aeration should be run. A t-test should be used to determine if the there is any significant effect of aeration.

Any questions on the comments can be addressed to me at (510) 412-2329.

cc: Jeff Rosenbloom, Chief
Wetlands and Sediment Management Section (W-3-3)

ROUTING AND TRANSMITTAL SLIP

Date 9/30

TO: (Name, office symbol, room number, building, Agency/Post)	Initials	Date
1. Allan Otte W-3-3		
2.		
3.		
4.		
5.		

	Action		File		Note and Return
	Approval		For Clearance		Per Conversation
	As Requested		For Correction		Prepare Reply
	Circulate	✓	For Your Information		See Me
	Comment		Investigate		Signature
	Coordination		Justify		

REMARKS

DO NOT use this form as a RECORD of approvals, concurrences, disposals, clearances, and similar actions

FROM: (Name, org. symbol, Agency/Post)	Room No.—Bldg.
Pat Young E-4	Phone No. 15-94

5041-102

OPTIONAL FORM 41 (Rev. 7-76)
Prescribed by GSA
FPMR (41 CFR) 101-11.206

* U.S. GPO: 1990 — 262-080

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

September 30, 1994

Steven L. Costa
Project Manager
CH2M Hill
P.O. Box 12681
Oakland, CA 94604-2681

Re: Third Bioassay Test of Ocean Disposed High-Strength Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

Dear Steve:

We have reviewed the two options proposed in your letter of September 14, 1994 for the timing of the third bioassay test required by the canneries' ocean disposal permits. We believe that information obtained during the different seasons would prove valuable. Thus, your proposal to change the schedule of the final bioassay test from December 1994 to June 1995 is approved. We understand that this will extend the term of the study beyond that stated in the permits. Since the modeling and evaluation will have been started on the first sets of data, we would expect to see the final study results by October 1995. As you know, the permits expire on August 31, 1996, and the canneries should reapply for permit renewal a few months prior to this expiration date. Because of the implications this report has for the designated ocean disposal site, we would like to receive the modeling and evaluation report with ample time to review it prior to the reapplication period.

Please call me at (415) 744-1594 if we need to discuss this further.

Sincerely,

Pat Young

Pat Young
American Samoa Program Manager
Office of Pacific Island and Native American Programs (E-4)

cc: Jim Cox, Van Camp Seafood Company
Norman Wei, StarKist Seafood Company
Tony Tausaga, American Samoa EPA
Sheila Wiegman, American Samoa EPA
Allan Ota, W-3-3
Amy Wagner, P-3-1

cc: Allan Ota

Engineers
Planners
Economists
Scientists

14 September 1993

OPE30702.MA

Mr. Norman L. Lovelace
Chief, Office of Pacific Island and
Native American Programs (E-4)
U.S. Environmental Protection Agency
Region IX
75 Hawthorne Street
San Francisco, CA 94105

Attention: **Patricia N.N. Young**
American Samoa Program Manager

Subject: Bioassay Testing of Ocean Disposed High-Strength Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

This correspondence is in response to your letter of August 29, 1994. I have asked Kurt Kline of Advanced Biological Testing, the bioassay laboratory we are using for this project, to review Amy Wagner's comments on the first round of testing. He will be able to incorporate all of her recommendations for the remaining bioassay tests. The testing schedule was delayed because of problems with one of the organisms, requiring the collection and shipping of additional samples and additional bioassay tests. We have scheduled the next (second) test for the first week in October, 1994.

The third and final test will be scheduled after the results of the second test have been reviewed, but no earlier than December 1994. However, there are two options available to do the third test: [1] do the third test by the end of 1994 and complete the modeling within the term of the study specified in the permits, or [2], if EPA believes seasonal results would be more valuable, we can extend the study to collect the final sample and do the final bioassay tests about next June (1995). This will extend the term of the study beyond that required by the permits. However, we are starting the modeling and evaluation based on the first set of data. Therefore, we could have near-final study results, using two bioassay tests, done within the term of the permits even if the third bioassay test is postponed. Please let me know which option you would prefer.

I hope you find the above response and explanations satisfactory. If you have any remaining questions please call me at 510-251-2426 (2251).

Thank you for your time and attention to this matter,

Sincerely,

CH2M HILL

Steven L. Costa
Project Manager

slc/epares.ltr
cc: Norman Wei/StarKist Samoa
James Cox/Van Camp Seafood Company, Inc.
Tony Tausaga/ASEPA
Sheila Wiegman/ASEPA
Mike Lee/USEPA
Allan Ota/USEPA (W-3-3)
Amy Wagner/USEPA (P-3-1)

7/13/94

To: ~~Mike Lee, OPINAP~~
Allan Ota, ~~Wetlands~~ and Sediment Management SEction

FROM: Pat Young, American Samoa Program Manager

Re: General Review of Ocean Disposal Permit Data, September 1993 to March 1994

Review covered three sets of data: 1) monthly analyses of individual waste streams (and volumes generated and disposed daily); 2) disposal logs; and, 2) monthly receiving water monitoring reports. To date we have received most reports from both canneries for September 1993 to April 1994. Only Samoa Packing has submitted March 1994 information. Review found the following:

Analyses of individual waste streams/volumes generated & disposed.

1. Individual waste stream analyses generally indicated concentrations within permit limits. SP had 14 exceedances, 7 of which were ammonia. Four exceedances (TS, TVSS, ammonia) were at least double the limits. (See attached handwritten review notes for details.)

StarKist had three exceedances of limit for TS, TVS and oil and grease for cooker juice in November 1993. USEPA was not notified by letter of exceedances.

Daily disposal logs.

1. Missing logs: 9/1-10/93; 10/1-8/93 from both SK and SP. No explanation given.

1. Nine of StarKist's February 1994 logs were missing either computer track printout or log sheet. Samoa Packing's logs were complete.

2. When boat captain changed in mid-February, ocean current direction which previously had been mostly SSW or WSW, changed to mostly SE. This raises the question of how is current direction being determined, and should we give guidance as it is not specified in permit. Also, under the new captain, based on recorded ocean direction and computer plot, **disposal operations occurred in the wrong quadrant on 10 trips in February and two trips in March.** (Disposal operations seemed to have been conducted correctly in remainder of March 1994 and previous months.)

3. Based on logs, rate of discharge exceeded twice, 2/16/-94: 137 gal/min/knot; 2/22/94: 123 g/m/k. (Limit for Dec. through May is 120 g/m/k.; June through Nov. is 140 g/m/k.)

4. Logs by Capt. Tracy indicated almost daily sightings of brown discharge, foam and/or sheen at disposal site prior to disposal operations. Other captains generally indicate no sightings.

Receiving water monitoring reports.

1. How is compliance determined? Need help in reviewing data.

2. Sample analyses received from Samoa Packing only for December 1993. Analyses received from StarKist for September, October, November 1993.

Items of note.

1. Canneries are sampling on-shore waste storage tanks twice/month to provide us with data to recalculate permit limits after 1 year's data for combined waste, rather than requiring limits/analyses on individual waste streams. Review of ammonia results indicate concentrations which are very high for StarKist, ranging from 2,000 mg/L to 10,800 mg/L, generally far above the highest existing permit limit of 1,830. Samoa Packing's results were generally within the highest permit limit of 3,470 mg/L. Any thoughts on why the high ammonia levels in the combined waste tank?

2. We have not been receiving computer disks with data in Lotus format from Samoa Packing.

Can we arrange to meet briefly within the next two weeks to discuss the above and how we want to deal with these items? At the least, I would like to send letters to the canneries requesting the information missing. Thanks.

Ocean Disposal Permit Data Review - Samoa Packing
Effective date 9/1/93

Sept. 1993 (No dish)

WASTE STREAMS

★ Exceedances - Sep. 1, 1993 Precooker Am: 940 (L=410)
" Press Am. 1430 (L=830)

Letter, 10/28/93 reported exceedances - no cause determined.

O.D. LOG

★ Only received 9/11-9/30/93. No explanation why other sept. logs missing.
OK

RECEIVING WATER MONITORING

OK (Narrative)

★ No analyses of monitoring station samples received

October, November, December 1993 (★ No dish)

WASTE STREAMS

★ Exceedances	10/93,	DAF	BOD:	400,000	(L: 349,350) ✓
	11/93	PC	O+G:	15,982	(L: 11,180) ✓
	12/93	PC	TS:	258,000	(L: 115,180) ✓
	12/93	PC	TVSS:	216,000	(L: 84,450) ✓
	12/93	PC	Amm:	1,120	(L: 410) ✓
	11/93	PW	TP:	3,400	(L: 2,950) ✓
	11/93	PW	Amm:	1,370	(L: 830) ✓

✓ letters sent re: exceedances

O.D. LOGS

See review of SK's submittal - generally ok

★ Oct. reports received only 10/11-31/93. Missing 10/1-10/8/93
No explanation why logs missing.

Receiving Water Monitoring

Narrative (ok)

★ No analyses of monitoring samples received for Oct, Nov.
(Received Dec. Report in May 1994 submittal)

Samoa Packing

January, February, March 1994 (★ No dish)

WASTE STREAMS

★ Exceedances 10/94 PC Amm. = 570 (L = 410)
" PW Amm. = 1180 (L = 830)
2/94 PW Amm = 3800 (L = 830)
3/94 PW. TS = 424,000 (L = 381,510)
3/94 PW BOD = 372,000 (L = 365,550)

Letters received re: exceedances

O.D. LOG

See review of StarKist logs (Jan, Feb 1994)
★ (problems in disposing in correct quadrant when captain changed)

MARCH 1994

★ 3/3/94 V#300 Current: SSE; Plot: NE (should be: NW)
(However current direction at end of dump = SSW)

★ 3/4/94 V#302 C = SE; P = NE (SB = NW)

Remainder of March ok

RECEIVING WATER MONITORING - ~~Rec'd 12/93, 1/94~~,

- Narrative ok (1/94, 3/94, 2/94)

- Samples received for 12/93, 1/94, 2/94 ★ Need to review

Reviewed July 1994

Ocean Disposal Permit - Star Kist

SEPTEMBER, OCT, NOV., DEC., JAN. FEB.

Waste Streams / Volumes

Nov. 93 Cooker Juice ★ TSS: Limit = 114,180; Analysis = 150,000
★ TVSS: L = 63,400; A = 118,150
★ O&G L = 11,810; A = 23,900
★ No correspondence re: notification of exceedances

Feb. 94 ~~Ted~~ Cooker Juice TVSS: Limit 63,400 / A = 66,000

Disposal Logs -

• Sept., Oct, Nov.

★ - Begins 9/11/93 - no earlier Sept. log - WHY NOT?
(Monitoring report of receiving waters is 9/10/93)

11/13/93? Current NW; initial dumping occurred in SE quadrant but mostly done in NE quadrant. No indication why changed.

11/25/93 Current NW; pattern on computer plot not oval; in south / SE quadrant - sort-of.

• DECEMBER 1993 - logs ok

StarKist (Disposal logs

2/14 Voyage #276 - NO log (log w/ SP)

2/16 Voyage 279 - no log (log w/ SP)

2/16 ✱ Rate of discharge = 137 gal/min/knot

2/21 ✱ Current = SE; ~~plot~~ boat plot was west; shmld have been NW

✱ New boat captain - ~~Ken~~ Lorn Len?

Is current correct - previous days currents were SSW or WSW

2/22/94 ✱ V#288 Current SW; disposal in NW quadrant - shmld be NE

2/22/94 ✱ V#289 Current SW; disposal in West quadrant, shmld be NE

✱123 = Rate of discharge

2/23/94 ✱ V#290 Current SE; D = West; SB = NW

✱ Current SE. D = West; SB = NW

2/24/94 V = 291 - ~~Plotter~~ Log missing (log w/ SP)

2/24/94 ✱ V = 292 Current SE, D = W, Shmld be NW

2/25/94 ✱ V = 293 Current SE, D = SW; Shmld be NW

2/25/94 ✱ V = 294 Current - SE; D = W, SB NW

2/26/94 ✱ V = 295 " "

2/28/94 V = 296 - ~~No plot~~ NO PLOT (plot w/ SP OK)

CORRECT!

When Darrell Tracy captain, logs indicated foam, sheen brown discharge, almost daily. With new captain no floatable mat'ls indicated when entered dump site.

Starkist

Disposal logs

January / February 1994

1/20/94 - new log sheet created ★ - relocated dumping pattern after wind shift (only should have done if current direction changed)

1/31 Windshift / disposal shift pattern (slight)
★ Brown discharge + sheen on water visible daily

2/1/94 No plot attached

(plot w/ Samoa Packing's submittal)

2/2/94 shift in disposal pattern due to wind shift

2/3/94 No plot attached. Note re: existence of prevailing current. (Plot w/ SP)

2/7/94 No plot attached - (Plot w/ SP)

2/9 Missing log (plot only) (Log w/ SP)

2/10 2nd trip (#271) missing log (Log w/ SP)

2/12 Fig 8 pattern on 2/11 + 2/12 - called locals

ROUTING AND TRANSMITTAL SLIP

Date 7/13/94

TO: (Name, office symbol, room number, building, Agency/Post)	Initials	Date
1. Allan Ota W-3-1		
2.		
3.		
4.		
5.		

	Action		File		Note and Return
	Approval		For Clearance		Per Conversation
	As Requested		For Correction		Prepare Reply
	Circulate	✓	For Your Information		See Me
	Comment		Investigate		Signature
	Coordination		Justify		

REMARKS

P/s. review - call me w/ your comments + recommendations. I believe Amy + Kurt Kline have been in contact throughout the tests + rec. dk w/ her. The LPC_{50} [redacted] original bioassay result used for dumps site modeling was 0.04%.

DO NOT use this form as a RECORD of approvals, concurrences, disposals, clearances, and similar actions

FROM: (Name, org. symbol, Agency/Post)	Room No.—Bldg.
Pat Young E-4	Phone No. 4594

5041-102

OPTIONAL FORM 41 (Rev. 7-76)
Prescribed by GSA
FPMR (41 CFR) 101-11.206

* U.S. GPO: 1990 — 262-080

Copy to Alan Ota, ASEPA

MEMORANDUM CH2MHILL

TO: Pat Young/USEPA

COPIES: Amy Wagner/USEPA (w/ attachments)
Kurt Kline/ABT (w/o attachments)

FROM: Steve Costa/CH2M HILL/SFO
Don Kingery/CH2M HILL/SFO

DATE: July 1, 1994

SUBJECT: Bioassay Testing of Starkist Samoa, Inc. and VCS Samoa Packing High Strength Waste

PROJECT: OPE030702.EL.R2

High strength waste (HSW) bioassays are required by Special Condition 3.3.5 of Starkist Samoa's and VCS Samoa Packing's ocean dumping permits. The results of the tests are presented in the attached: "*Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa*" prepared by Advanced Biological Testing Inc., Tiburon, California.

Acute effluent bioassays were conducted on *Mysidopsis bahia* (mysid shrimp) juveniles, *Mytilus edulis* (blue mussel) larvae, *Strongylocentrotus purpuratus* (purple sea urchin) larvae, and *Citharichthys stigmaeus* (speckled sanddab) juveniles using HSW collected separately from the Starkist Samoa and VCS Samoa Packing canneries in Pago Pago Harbor, American Samoa. The results of these bioassays are summarized in the table below.

Based on the results of the bioassays, we recommend the following changes to the HSW bioassay protocol:

Reduce the upper end of the HSW concentration series for all bioassays to a maximum of 3.0%. The results of the bioassay tests give a better understanding of the test concentrations needed. No additional information is required at concentrations greater than 3.0%. Reducing the maximum concentrations will reduce the amount of HSW that needs to be sampled and shipped. We recommend a series of concentrations for the bioassays of 3.0%, 1.5%, 0.8%, 0.2%, 0.1%, and 0.05%.

Continue running bioassays with *Mytilus edulis* while monitoring the effects of aeration on organism mortality but drop the use of *Strongylocentrotus purpuratus* larvae as test organisms for the HSW. This recommendation is made for the following reasons:

- Special Condition 3.3.5 of the permits requires only three organisms be tested; one organism each out of three specified groups. *Mysidopsis bahia*

and *Citharichthys stigmaeus* satisfy the requirements for Groups 2 and 3. Group 1 contains larval stages of both bivalves and echinoderms and running just *Mytilus edulis* should satisfy this requirement.

- Because of the high oxygen demand of the effluent, all test containers required aeration throughout the tests to maintain adequate oxygen concentrations for the test organisms. Aerating the chambers using *Mytilus edulis* and *Strongylocentrotus purpuratus* larvae as bioassay test organisms gives problematic results. Aeration is standard protocol for bioassays on fish and invertebrates when oxygen levels fall below 40% of saturation, but is not standard protocol for bioassays on larval bivalves and echinoderms. The effects of aerating the water on the survival of these organisms is not known. Because the *Mytilus edulis* bioassays are only run for two days (vs. four for the *Strongylocentrotus purpuratus*) the organisms are exposed for half the time and the effects of aeration may be reduced.

- The mortality of the control group was substantial for the echinoderms and is unacceptable according to protocol. The cause of the high mortality in the control is not known at this time.

Please review the above recommendations. We suggest Amy Wagner contact Kurt Kline, Advanced Biological Testing Inc., directly at (415)435-7878 to discuss any comments you have on the bioassay protocols.

Summary of High Strength Waste Bioassay Results.

Test Organism	Starkist Samoa		VCS Samoa Packing	
	LC_{50}	NOEC/IC_{50} [1]	LC_{50}	NOEC/IC_{50} [1]
Citharichthys stigmaeus (sanddab)	0.27%	0.2%	0.59%	0.4%
Mysidopsis bahia (mysid shrimp)	0.12%	0.05%	0.59%	0.05%
Mytilus edulis (blue mussel)	> 1.2%	< 0.08%	> 1.2%	< 0.08
Strongylocentrotus purpuratus [2] (urchin)	> 1.2%	< 0.08%	> 1.2%	0.1%

[1] NOEC reported for the juvenile sanddabs and mysid shrimp, IC_{50} reported for the mussel and urchin larvae.

[2] Control survival of 64.4% is unacceptable according to protocol.

RESULTS OF A BIOASSAY CONDUCTED ON TWO HIGH STRENGTH WASTE SAMPLES FROM THE VAN CAMP AND STARKIST TUNA CANNERIES IN AMERICAN SAMOA

Advanced
Biological
Testing Inc.

RESULTS OF A BIOASSAY CONDUCTED ON TWO HIGH STRENGTH WASTE SAMPLES FROM THE VAN CAMP AND STARKIST TUNA CANNERIES IN AMERICAN SAMOA

Prepared for:

CH2M Hill California, Inc.
1111 Broadway
Oakland, CA 94607
Project # PDX 30702

Prepared by:

Advanced Biological Testing Inc.
98 Main St., # 419
Tiburon, Ca. 94920

June 29, 1994

Ref: 9309-2

1.0 INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted acute effluent bioassay testing on *Mysidopsis bahia*, *Mytilus edulis*, *Strongylocentrotus purpuratus* and *Citharichthys stigmaeus* using high strength wastes (HSW) collected separately from the Van Camp (HSW-1) and Starkist (HSW-2) tuna canneries in American Samoa. The study was run using methods generally specified in EPA 1991 and in a Sampling and Testing Plan submitted to the EPA.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.0 METHODS

2.1 EFFLUENT SAMPLING

The high strength wastes were sampled as composites on February 16, 1994 by personnel from CH2M Hill. Due to shipping and airline scheduling problems, frequently encountered in this region, the sample was received by the laboratory on February 19, 1994. Two five gallon carboys were provided from each cannery defined as HSW-1 (VCS) and HSW-2 (SK) and were maintained in ice-filled coolers from the date of sampling until laboratory receipt. The sample were at 2-3°C upon receipt.

Due to the test failure in the echinoderms, both of the HSW were resampled on March 30, 1994, and shipped to ABT arriving on April 4, 1994.

2.2 SAMPLE PREPARATION

2.2.1 Testing on the speckled sanddab, *Citharichthys stigmaeus*

After extensive discussions with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at eight concentrations starting from 3.0% and dropping using a 50% dilution factor. The final concentrations were 3.0, 1.5, 1.25, 0.8, 0.4, 0.2, 0.1 and 0.05% as vol:vol dilutions in seawater. The diluent was filtered seawater from the Bodega Bay Marine Laboratory. The dilutions were brought up to the test temperature (14°C) and aerated continuously. Based upon data provided by CH2M Hill, and subsequently supported by information from the EPA, these effluents have an extremely high biological oxygen demand, therefore aeration was carried out from the beginning of the test.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 25, 12.5, 6.25, 3.1, and 1.6 mg/L in 30 ppt seawater in a 24 hour test.

2.2.2 Testing on the mysid, *Mysidopsis bahia*

Both of the high strength wastes were tested twice, once in a concentration series of 25, 12.5, 6.25, 3.1, 1.6, 0.8, and 0.4% vol:vol in seawater, and after discussions with the EPA, a second

time at a lower concentration series of 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05% vol:vol dilutions. The diluent was filtered seawater from the Bodega Bay Marine Laboratory. The dilutions were brought up to the test temperature (20°C) and aerated continuously.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 20, 10, 5, 2.5 and 1.25 mg/L in 30 ppt seawater in a 96 hour test.

2.2.3 Echinoderm and Bivalve Larval Bioassay

Test solutions used in the bioassays were prepared using San Francisco Bay seawater at 28 ppt in serial dilution (0.5) to create 0.08%, 0.15%, 0.3%, 0.6% and 1.2% test concentrations for the bioassays. The echinoderm test failed control survival in two testing attempts using the initial HSW delivered on February 19, 1994. A second sample was requested from each cannery which was delivered on April 4, 1994. The echinoderm test again marginally failed the controls and the results of the study are presented for information. The bivalve study conducted concurrently with the echinoderm bioassay passed the control criteria.

The reference toxicant for the echinoderm and bivalve larval bioassays was copper at test concentrations of 0.56, 3.2, 10, 32, and 56 µg/L.

2.2.4 *Citharichthys stigmaeus*

The bioassays were carried out on juvenile *Citharichthys stigmaeus*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on February 19, 1994. The test conditions are summarized in Table 1. Five replicates of each concentration were tested with ten juvenile fish per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.5 *Mysidopsis bahia*

The first bioassay was carried out on 7-10 day old larval *Mysidopsis bahia*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on February 19, 1994. The test conditions for this test are summarized in Table 2. The second test was carried out on larval mysids supplied by Aquatox from Hot Springs, Arkansas. The animals

were received at ABT on February 26, 1994. The test conditions for the second test are summarized in Table 3.

Five replicates of each concentration were tested with ten larval mysids per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.6 Echinoderm Larval Development Test

The echinoderm larvae survival and development test followed draft ASTM methods (ASTM, 1994). Purple urchins, *Strongylocentrotus purpuratus,* were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by intercoelomic injection of 0.5M KCl. Released eggs were placed in individual containers of filtered seawater, and sperm was collected dry and held on ice. Gametes were mixed and allowed to fertilize for up to two hours. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 µm. Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to an initial density of 15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at 16 ± 2°C under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0, 24, 48 and 72 hours in water quality replicates. Total ammonia was measured in the 1.2% sample at 0 and 48 hours. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

2.2.7 *Mytilus edulis* Larval Survival and Development Test

The bivalve larvae survival and development test was run in parallel with the echinoderm using the second set of effluents. The test followed methods in ASTM (1993). Bay mussels, *Mytilus edulis,* were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by heat shocking. Released gametes were placed in individual containers of filtered seawater and examined for viability. Gametes were mixed and allowed to fertilize for up to two hours, under gentle aeration. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 µm. Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to an initial density of

15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at 16 ± 2°C under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0 and 48 hours; temperature was also recorded at 24 hours. Total ammonia was measured in 1.2% sample at 0 and 48 hours. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

Dissolved oxygen levels of test solutions of HSW-2 fell below 60% saturation in both the bivalve and echinoderm tests. Gentle aeration was started on Day 1, and continued for the duration of the tests. To assess the effects of aeration, control replicates 4 and 5 were aerated beginning on Day 1 for both the bivalve and echinoderm tests. No statistical differences were observed between aerated and unaerated control replicates.

2.3 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and TU values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis.

At the conclusion of the echinoderm tests, data were evaluated statistically to estimate the LC50 and IC50 values for the elutriate tests. The LC50 and IC50 values were estimated using the Probit or the Linear Interpolation (Bootstrap) Method.

The LC50 and the IC50 for the bivalve larvae copper reference toxicant test were both within two standard deviations of the laboratory means of 26.3 µg/L and 8.9 µg/L, respectively, indicating normal sensitivity of the test organisms. No laboratory means for the echinoderm larvae copper reference toxicant test have yet been established.

Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if available.

The NOEC (No Observable Effect Concentration) is the highest tested concentration at which mortality is not significantly different from the control.

3.0 RESULTS

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at 20 ± 2°C; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected at approximately 1 hour after test initiation in all of the concentration even with supplemental aeration therefore aeration was maintained in all chambers for the duration of the test. Ammonia was measured in two replicates from each concentration daily and was a potentially significant toxic component of the test for all concentrations.

3.1 *Citharichthys stigmaeus*

The LC50 for HSW-1 was 0.59%. Mortality in the effluent was rapid at the highest concentrations, occurring in 2-4 hours. There was significant mortality at 3.0, 1.5, and 0.8% concentrations compared to the control at 96 hours. The NOEC was 0.4% and the LOEC was 0.8%

The LC50 for HSW-2 was 0.27%. Mortality in the effluent was rapid at the highest concentrations, generally occurring in 2-4 hours. There was significant mortality at 3, 1.5, 0.8 and 0.4% concentrations compared to the control at 96 hours. The NOEC was 0.2%, and the LOEC was 0.4%.

The reference toxicant test required the use of the Trimmed Spearman-Karber method and generated an LC50 of 4.34 mg/L, an NOEC of 3.1 mg/L, and an LOEC of 6.25 mg/L. This is the first reference toxicant test on *Citharichthys* at this laboratory, therefore no database has been established by this laboratory.

3.2 *Mysidopsis bahia*

The LC50 results for both HSW effluents in the initial tests were <0.4%. Based upon the fact that no definitive LC50 could be calculated, the tests were rerun as described in the methods.

The LC50 for HSW-1 was 0.59%. Mortality in the 1.6% and 0.8% effluent was incomplete at 24 hours. At 96 hours, there was significant mortality at 1.6, 0.8, 0.4, and 0.1% concentrations compared to the control. The NOEC was 0.05% and the LOEC was 0.1%.

In the second test series the LC50 for HSW-2 was 0.12%. Mortality in the 1.6% and 0.8% effluent was complete at 24 hours. There was significant mortality at 96 hours in the 1.6, 0.8, 0.4, 0.2 and 0.1% concentrations compared to the control. The NOEC was 0.05%, and the LOEC was 0.1%.

The reference toxicant test had an LC50 of 8.90 mg/L, with an NOEC of <1.25 mg/L and an LOEC of 1.25 mg/L. This is the first reference toxicant test on *Mysidopsis* at this laboratory, therefore no database has been established.

3.3 ECHINODERM LARVAL BIOASSAY

Control survival was marginal and unacceptable according to the protocol at 64.4% with 5.7% abnormal development. Total survival was relatively high and equal to control survival in all concentrations, however all of the embryos were abnormally developed at 0.15% to 1.2% in HSW-1 and from 0.08% to 1.2% in HSW-2. The LC50 for both effluents was greater than 1.2% however the IC50 was 0.1% for HSW-1 and <0.08% for HSW-2.

The reference toxicant analysis yielded an LC50 of 11.8 µg/L and an IC50 of 10.1 µg/L. The use of the echinoderm larval bioassay is still limited and no data is available for comparison.

3.4 BIVALVE LARVAL BIOASSAY

Control survival was acceptable at 98.1% with 6.3% abnormal development. Total survival was relatively high in all concentrations, however all of the embryos were abnormally developed at 0.15% to 1.2% in HSW-1 and HSW-2. The LC50 for both effluents was greater than 1.2% however the IC50s were <0.08% for both HSW-1 and HSW-2.

The LC50 and IC50 for the bivalve larvae copper reference toxicant test were both within two standard deviations of the laboratory means of 26.3 µg/L and 8.9 µg/L, respectively, indicating normal sensitivity of the test organisms.

3.5 AMMONIA MEASUREMENTS

Ammonia in both of the HSW was very high. When measured in a 25% dilution in seawater, ammonia levels ranged from 160 to 180 mg/L. If converted to the 100% concentration, the

ammonia level would be above 640 mg/L. Tested concentrations in the *Citharichthys* bioassay ranged from 0.08 to 0.17 mg/L in the lowest concentration (0.05%) to 3.44 to 9.65 mg/L in the 3.0% dilution. At each test concentration, HSW-2 generated the higher ammonia levels. The toxicity of ammonia to sanddabs is well documented and the measured levels in the three highest concentrations in HSW-2 and the two highest concentrations in HSW-1 were sufficient to cause toxicity in the test animals in 24 hours. The mysid test results appear to indicate a slightly higher tolerance to ammonia as has been shown in the literature.

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TABLE 1

Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Citharichthys stigmaeus* (U.S. EPA 1991)

Parameter	Data
Test Species	*Citharichthys stigmaeus*
Supplier	J. Brezina and Associates
Collection location	Tomales Bay
Date Acquired	2/19/94
Acclimation Time	24 hours
Acclimation Water	30 ppt seawater
Acclimation Temperature	15±2°C
Age group	Juveniles, 3-5 cm TL
Sample Identification	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	96 hour static acute, renewal at 48 hours
Test Dates	2/19/94 to 2/23/94
Control Water	Bodega Bay seawater
Test Temperature	15 ± 1°C
Test Photoperiod	16 L : 8 D
Initial Salinity	30 ± 2 ppt
Test Chamber	20 L polyethylene chamber
Animals/Replicate	10 animals/replicate
Exposure Volume	5 L
Replicates/Treatment	5
Feeding	None
Deviations from procedures	Due to aeration, salinity increased throughout test.

TABLE 2

Bioassay Procedure And Organism Data For the Survival Bioassay Using *Mysidopsis bahia* (U.S. EPA 1991)

Parameter	Data
Test Species	*Mysidopsis bahia*
Supplier	J. Brezina and Associates
Date Acquired	2/19/94
Acclimation Time	overnight
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	larvae
Sample Identification	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	2/19/94 to 2/23/94
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	25 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Due to aeration, salinity increased throughout test

TABLE 3

Bioassay Procedure And Organism Data For the Survival Bioassay Using *Mysidopsis bahia* (U.S. EPA 1991)

Parameter	Data
Test Species	*Mysidopsis bahia*
Supplier	Aquatox
Date Acquired	2/26/94
Acclimation Time	Overnight
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	larvae
Sample Identification	
Sample ID(s)	940219-1, -2
Date Sampled	2/16/94
Date Received at ABT	2/19/94
Volume Received	Ten gallons
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	2/27/94 to 3/2/94
Control Water	Bodega Bay seawater
Test Temperature	20 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	25 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	Due to aeration, salinity increased throughout test

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TABLE 4

Bioassay Procedure And Organism Data For The Bioassay Using Larvae of *Strongylocentrotus purpuratus* (modified ASTM 1994)

Parameter	Data
Test Species	*Strongylocentrotus purpuratus*
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	4/7/94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
Sample Identification	
Sample ID(s)	940404-3, -4
Date Sampled	3/30/94
Date Received at ABT	4/4/94
Volume Received	Two liters
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute/static; 96 hours
Test Dates	4/7/94 to 4/11/94
Control Water	San Francisco Bay seawater, 0.45 µm filtered and uv-sterilized
Test Temperature	16 ± 2°C
Test Photoperiod	14 L : 10 D
Salinity	30 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	5
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 5

Bioassay Procedure And Organism Data
For The 48 Hour Bioassay
Using Larvae of *Mytilus edulis* (ASTM 1993)

Parameter	Data
Test Species	*Mytilus edulis*
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	4/7/94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
Sample Identification	
Sample ID(s)	940404-3,-4
Date Sampled	3/30/94
Date Received at ABT	4/4/94
Volume Received	Two liters
Sample Storage Conditions	4°C in the dark
Test Procedures	
Type; Duration	Acute; static; 48 hours
Test Dates	4/7/94 to 4/9/94
Control Water	San Francisco Bay seawater, 0.45 µm filtered and uv-sterilized
Test Temperature	16 ± 2°C
Test Photoperiod	14 L : 10 D
Salinity	30 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	3
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 6

SUMMARY OF RESULTS FOR THE HIGH STRENGTH WASTE BIOASSAYS

Species	Test	Endpoint	HSW-1	HSW-2
Citharichthys stigmaeus	96 hr static	LC50	0.59%	0.27%
Mysidopsis bahia	96 hr static	LC50	0.59%	0.12%
Strongylocentrotus purpuratus	96 hr static	LC50	>1.2%	>1.2%
		IC50	0.10%	<0.08%
Mytilus edulis	48 hr static	LC50	>1.2%	>1.2%
		IC50	<0.08%	<0.08%

Note:
HSW-1: Van Camp
HSW-2: Starkist

TABLE 7

SUMMARY OF RESULTS FOR THE REFERENCE TOXICANT (S.D.S.) TEST

Citharichthys stigmaeus

Concentration (mg/L)	% Survival	ECp (mg/L)		NOEC (mg/L)	LOEC (mg/L)
Control	93.3	EC50	4.3449	3.1	6.25
1.6	80.0				
3.1	100.0				
6.2	0.0				
12.5	0.0				
25	0.0				

Mysidopsis bahia

Concentration (mg/L)	% Survival	ECp (mg/L)		NOEC (mg/L)	LOEC (mg/L)
Control	90.0	EC50	8.90 (3.04-69.22)	<1.25	1.25
1.25	70.0				
2.5	56.7				
5	46.7				
10	46.7				
20	36.7				

* Statistically significant.
ICp/LCp: Inhibition/Lethal Concentration for p% of the organisms.
NOEC: No Observable Effect Concentration.
TU: 100%/NOEC.

4.0 REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.

ASTM. 1993. Annual Book of Standards. Vol. 11.04. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve mollusca. E-724-89.

ASTM. 1994. Annual Book of ASTM Standards Vol. 11.04. Guide for conducting static acute toxicity tests with echinoid embryos. Proposed Standard in review.

APPENDIX A

ANALYTICAL DATA

APPENDIX TABLE 1

SAMPLE WATER QUALITY

Date	Day	Sample	pH (units)	DO (mg/L)	Total NH3 (mg/L)	Initial Salinity (ppt)
4/7/94	0	HSW-1, 1.2%	7.62	8.0	62.5	26
	0	HSW-2, 1.2%	6.87	7.9	51.6	26
4/9/94	2	HSW-1, 1.2%	-	-	26.4	-
	2	HSW-2, 1.2%	-	-	41.2	-
4/11/94	4	HSW-1, 1.2%	-	-	33.5	-
	4	HSW-2, 1.2%	-	-	41.9	-

APPENDIX TABLE 2

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	6.2	0.02	14.0	32.0	8.07	5.5	0.01	13.2	31.5	8.08	5.5		13.8	32.9	8.03	6.0		14.0	35.0	8.06	6.1	0.02	14.4	36.0
	2						8.11	5.8		13.7	31.0	8.13	5.6	0.12	14.2	31.7	8.12	6.0		14.3	33.0	8.13	6.1		15.0	33.0
	3						8.10	6.0		13.8	30.9	8.12	5.7		14.2	31.8	8.11	6.0		14.4	32.0	8.12	5.8		15.2	33.0
	4						8.10	6.0		13.2	31.6	8.13	5.7		13.6	33.1	8.11	6.0	<0.10	13.9	35.0	8.13	5.6		14.6	36.0
	5						8.10	6.0		13.3	31.7	8.12	5.6		13.9	33.3	8.12	6.0		14.0	34.0	8.13	5.8		14.7	37.0
0.05	1	8.00	6.3	0.19	14.0	32.2	8.04	6.0	0.08	13.5	33.8	8.07	5.6		13.9	36.2	8.07	6.0		14.0	38.0	8.07	5.8	0.10	14.8	40.0
	2						8.03	6.0		13.6	33.8	8.07	5.5	0.05	13.9	36.4	8.04	6.0		14.1	38.0	8.06	5.6		14.7	40.0
	3						8.05	6.0		13.5	32.7	8.10	5.5		14.1	33.6	8.08	6.0		14.2	35.0	8.10	5.6		14.6	35.0
	4						8.01	6.0		13.5	32.3	8.07	5.6		14.1	33.4	8.06	6.0	<0.10	14.2	34.0	8.04	5.8		14.7	35.0
	5						8.05	5.9		13.6	33.1	8.09	5.6		14.1	34.1	8.09	6.0		14.2	35.0	8.10	5.8		14.9	36.0
0.1	1	8.01	6.2	0.25	14.0	32.1	8.06	6.0	0.13	13.5	31.8	8.12	5.6		13.9	32.6	8.11	6.0		14.1	34.0	8.13	5.8	0.12	14.9	34.0
	2						8.03	5.9		13.8	31.7	8.10	5.7	0.08	14.2	32.6	8.10	6.0		14.4	33.0	8.10	5.8		14.9	34.0
	3						8.01	5.8		13.3	32.8	8.08	5.7		13.8	34.8	8.06	5.9		14.0	37.0	8.06	5.6		14.4	39.0
	4						8.04	5.9		13.8	32.6	8.12	5.8		14.5	33.9	8.11	6.0	<0.10	14.6	35.0	8.11	5.7		14.9	36.0
0.2	1	8.01	6.0	0.54	14.0	32.1	8.04	5.7	0.20	14.2	30.0	8.14	5.9		14.4	31.1	8.13	6.0		14.3	32.0	8.13	6.0	0.17	14.9	34.0
	2						8.01	5.8		14.1	29.9	8.14	5.8	0.17	14.5	30.5	8.16	6.0		14.6	31.0	8.16	5.9		14.9	32.0
	3						7.98	5.8		13.9	29.8	8.12	5.8		14.2	30.3	8.13	5.9		14.9	31.0	8.14	5.9		15.0	32.0
	4						8.02	5.8		13.9	29.8	8.15	5.8		14.2	30.5	8.15	6.3	NT	14.9	31.0	8.16	5.8		15.0	32.0
	5						8.03	5.8		13.8	29.8	8.13	5.8		14.2	30.5	8.15	6.3		14.9	31.0	8.17	5.8		15.0	32.0
0.4	1	7.93	6.1	0.89	14.0	32.0	7.95	5.4	0.33	13.7	30.1	8.12	5.4		14.2	30.8	8.14	6.3		14.3	32.0	8.17	5.8	0.31	15.0	32.0
	2						7.98	5.6		14.4	30.2	8.13	5.8	0.25	14.8	31.1	8.17	6.3		14.9	32.0	8.18	5.8		14.7	33.0
	3						8.00	5.9		14.4	30.2	8.15	5.7		14.3	31.6	8.18	6.3		14.6	33.0	8.06	5.8		14.6	34.0
	4						7.76	4.6		14.0	29.9	8.06	5.8		14.5	30.3	8.09	6.2	0.17	14.7	31.0	8.11	5.8		14.6	36.0
	5						7.93	5.2		13.5	30.4	8.11	5.6		14.0	31.4	8.13	6.2		14.0	32.0	8.19	5.6		14.3	34.0
0.8	1	7.68	6.1	2.01	14.0	32.0	7.89	5.2	0.64	13.7	30.8	8.15	5.6		14.1	31.7	8.15	6.2		14.2	33.0	8.10	5.8	0.51	14.7	33.0
	3						7.82	5.1		13.1	31.2	8.09	5.6	0.40	13.7	32.6	8.06	6.3		13.90	34.0	8.10	5.8		14.20	36.0
	4						7.95	5.4		14.1	30.8	8.16	5.5		14.5	32.0	8.17	6.4	0.48	14.3	34.0	8.18	5.8		14.4	35.0
	5						7.88	5.4		13.2	31.5	8.13	5.7		14.5	32.7	8.16	6.3		14.5	34.0	8.21	5.8		14.3	35.0
1.5	1	7.51	6.0	3.56	14.0	32.2	7.83	5.2	1.43	13.3	32.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.76	4.8		13.5	31.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.75	5.0		12.9	32.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.76	5.2		12.9	32.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.76	5.1		12.9	32.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3.0	1	7.23	5.9	11.1	14.0	32.1	7.85	5.6	3.44	13.6	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.74	4.6		13.9	33.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.81	5.0		13.9	33.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.75	4.7		14.1	33.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.81	5.0		19.2	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		7.23	5.9	0.02	14.0	32.0	7.74	4.6	0.01	12.9	29.8	8.06	5.4	0.05	13.6	30.3	8.03	5.9	<0.10	13.9	31.0	8.04	5.6	0.02	14.2	32.0
Max		8.02	6.3	11.1	14.0	32.2	8.11	6.0	3.44	19.2	33.8	8.16	5.9	0.40	14.8	36.4	8.18	6.4	0.48	14.9	38.0	8.21	6.1	0.51	15.2	40.0

Note: — = All animals dead.
NT = Not taken.
0.1 replicate 5 not stocked.
0.8 replicate 2 lost due to lab error.

APPENDIX TABLE 2 (Cont'd)

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	6.2	0.02	14.0	32.0	8.08	5.5	0.01	13.2	31.5	8.02	5.5		13.8	32.9	8.03	6.0		14.0	35.0	8.06	6.1	0.02	14.4	36.0
	2						8.11	5.8		13.7	31.0	8.13	5.6	0.12	14.2	31.7	8.12	6.0		14.3	33.0	8.13	6.1		15.0	33.0
	3						8.10	6.0		13.8	30.9	8.12	5.7		14.2	31.8	8.11	6.0		14.4	32.0	8.12	5.8		15.2	33.0
	4						8.10	6.0		13.2	31.6	8.13	5.7		13.6	33.1	8.11	6.0	<0.10	13.9	35.0	8.13	5.6		14.6	36.0
	5						8.10	6.0		13.3	31.7	8.12	5.6		13.9	33.3	8.12	6.0		14.0	34.0	8.13	5.8		14.7	37.0
0.05	1	7.89	6.1	0.32	14.0	32.0	7.98	6.0		13.5	36.2	8.02	5.6		13.9	41.1	8.02	6.4		14.0	38.0	8.03	5.2	0.13	14.4	40.0
	2						8.03	6.2	0.17	14.5	34.0	8.11	5.6	0.12	15.0	35.4	8.13	6.4		15.2	38.0	8.15	5.6		15.2	40.0
	3						8.01	6.0		13.6	33.7	8.05	5.7		14.1	34.9	8.10	6.3		14.4	36.0	8.10	5.6		14.2	37.0
	4						8.02	6.0		13.3	34.5	8.04	5.8		13.7	36.9	8.07	6.3	<0.10	13.9	38.0	8.06	5.6		14.0	40.0
	5						8.01	6.0		13.3	34.5	8.04	5.6		13.8	36.5	8.05	6.3		14.0	38.0	8.06	5.6		14.0	40.0
0.1	1	7.96	6.0	0.56	14.0	32.2	8.02	6.1		13.3	35.0	8.03	5.4		13.7	37.8	8.04	6.2		13.9	40.0	8.06	5.8	0.12	13.9	40.0
	2						8.03	6.1	0.24	14.2	33.6	8.09	5.5	0.13	14.9	34.5	8.11	6.3		14.9	35.0	8.13	5.8		14.6	36.0
	3						8.02	6.0		13.8	34.2	8.05	5.7		14.2	36.1	8.06	6.3		14.4	38.0	8.08	5.8		14.3	40.0
	4						8.02	5.9		14.3	33.5	8.07	5.5		14.9	34.2	8.09	6.3	<0.10	15.0	35.0	8.11	5.8		14.7	36.0
	5						8.04	6.1		13.2	33.6	8.07	5.6		14.8	34.4	8.11	6.3		14.0	35.0	8.13	5.8		13.9	36.0
0.2	1	7.87	6.1	1.32	14.0	32.0	8.03	6.0		13.2	33.5	8.11	5.6		13.9	34.3	8.12	6.3		14.1	35.0	8.15	5.8	0.20	13.8	36.0
	2						8.02	6.0	0.53	13.2	33.6	8.10	5.7	0.20	13.9	34.6	8.12	6.3		14.1	35.0	8.14	5.8		13.7	37.0
	3						8.03	6.0		13.5	33.5	8.10	5.8		14.1	34.1	8.13	6.3		14.3	35.0	8.15	5.8		13.9	36.0
	4						8.01	6.0		13.5	33.7	8.09	5.8		14.0	34.8	8.12	6.3	0.22	14.3	36.0	8.14	5.8		13.9	37.0
	5						8.02	6.0		13.8	33.8	8.10	5.7		14.2	34.8	8.04	6.3		14.3	35.0	8.15	5.8		14.2	36.0
0.4	1	7.66	6.0	3.00	14.0	32.1	7.95	5.8		13.2	35.1	7.99	5.4		13.8	38.2	8.08	6.3		13.9	41.0	8.05	5.8	0.30	13.7	40.0
	2						7.97	5.8	0.86	13.2	34.5	8.06	5.3	0.32	13.9	36.3	8.10	6.3		14.1	38.0	8.08	5.8		13.7	41.0
	3						7.99	6.0		14.5	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.99	5.9		14.4	33.5	7.89	5.1		15.0	34.1	—	—	—	—	—	—	—	—	—	—
	5						7.99	5.9		14.4	33.6	8.04	5.4		14.8	34.5	8.13	6.3	0.23	14.9	35.0	8.15	5.8		15.2	36.0
0.8	1	7.35	6.0	6.34	14.0	32.0	7.88	5.4		13.5	35.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.93	5.7	1.95	14.1	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.91	5.7		13.9	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.93	5.7		13.9	33.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.92	5.8		14.2	33.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.5	2	7.00	5.9	14.6	14.0	32.0	7.84	5.5		14.1	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.80	5.4	4.23	14.2	33.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.85	5.4		13.9	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.85	5.4		13.9	33.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3.0	1	6.81	5.7	28.5	14.0	32.0	7.89	5.7		13.9	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.86	5.9	9.65	13.8	33.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.88	5.9		13.6	33.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.81	5.8		13.0	34.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.81	5.8		12.9	34.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.81	5.7	0.02	14.0	32.0	7.80	5.4	0.17	12.9	30.9	7.89	5.1	0.12	13.6	31.7	8.02	6.0	<0.10	13.9	32.0	8.03	5.2	0.12	13.7	33.0
Max		8.02	6.2	28.50	14.0	32.2	8.11	6.2	9.65	14.5	36.2	8.13	5.8	0.32	15.0	41.1	8.13	6.4	0.23	15.2	41.0	8.15	6.1	0.30	15.2	41.0

Note: — = All animals dead.

APPENDIX TABLE 3

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.05	1	10	10	10	10	10	100	
	2	10	10	9	9	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	98.0
0.1	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	97.5
0.2	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	9	9	90	98.0
0.4	1	10	10	10	10	10	100	
	2	10	7	6	6	6	60	
	3	10	10	8	8	8	80	
	4	10	9	9	9	9	90	
	5	10	10	9	9	9	90	84.0
0.8	1	10	5	3	3	1	10	
	3	10	10	9	9	9	90	
	4	10	9	1	1	0	0	
	5	10	5	5	3	3	30	32.5
1.5	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
30	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Notes: — = All animals dead.

APPENDIX TABLE 3 (Cont'd)

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	100.0
0.05	1	10	10	10	10	9	90	
	2	10	10	10	10	9	90	
	3	10	10	10	10	10	100	
	4	10	10	10	10	9	90	
	5	10	10	10	10	10	100	94.0
0.1	1	10	10	10	9	9	90	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	98.0
0.2	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	9	9	9	90	
	5	10	10	9	9	9	90	96.0
0.4	1	10	4	3	2	2	20	
	2	10	4	3	3	2	20	
	3	10	0	—	—	—	0	
	4	10	3	0	—	—	0	
	5	10	3	3	3	3	30	14.0
0.8	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
1.5	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
3	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Notes: — = All animals dead.

APPENDIX TABLE 4

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration		Day 0				Day 1			
(mg/L)	Rep	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	**1**	8.02	5.8	15.9	32	7.20	5.7	15.2	31
	2					7.31	5.0	15.1	31
	3					7.31	4.7	15.1	31
1.6	**1**	8.03	5.8	15.9	32	7.49	4.7	15.1	31
	2					7.52	4.2	15.1	31
	3					7.51	4.1	15.2	31
3.1	**1**	8.03	5.8	15.9	32	7.49	4.0	15.1	31
	2					7.43	4.0	15.2	30
	3					7.51	3.9	15.1	31
6.25	**1**	8.03	5.8	15.9	32	7.49	4.1	15.1	31
	2					7.48	4.1	15.1	30
	3					7.47	4.0	15.1	31
12.5	**1**	8.04	5.8	15.9	32	7.40	3.9	15.1	31
	2					7.44	3.7	15.1	31
	3					7.51	3.7	15.1	31
25	**1**	8.03	5.7	15.9	32	7.44	3.0	15.1	31
	2					7.42	3.1	15.1	31
	3					7.36	3.2	15.0	31
Min		8.02	5.7	15.9	32	7.20	3.0	15.0	30
Max		8.04	5.8	15.9	32	7.52	5.7	15.2	31

APPENDIX TABLE 5

Citharichthys stigmaeus
SURVIVAL DATA
FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	% Survival	Average % Survival
Control	1	5	4	80	
	2	5	5	100	
	3	5	5	100	93.3
1.6	1	5	2	40	
	2	5	5	100	
	3	5	5	100	80.0
3.1	1	5	5	100	
	2	5	5	100	
	3	5	5	100	100.0
6.25	1	5	0	0	
	2	5	0	0	
	3	5	0	0	0.0
12.5	1	5	0	0	
	2	5	0	0	
	3	5	0	0	0.0
25	1	5	0	0	
	2	5	0	0	
	3	5	0	0	0.0

APPENDIX TABLE 6

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.06	5.4		18.0	32.0	8.14	5.2	<0.01	19.6	32.0	8.11	5.1		19.8	33.0	8.11	4.6	<0.10	21.7	33.9	8.08	4.9	<0.10	21.1	34.1
	2						8.13	5.2		19.9	32.0	8.08	5.2		20.1	33.0	8.07	4.6		21.6	33.6	8.07	5.1		21.1	34.1
	3						8.16	5.1		19.7	32.0	8.12	5.4		20.2	33.6	8.11	4.5		21.6	34.7	8.09	5.1		21.1	34.0
	4						8.16	5.2		19.7	32.0	8.12	5.4		20.2	33.3	8.14	4.5		21.6	33.9	8.12	5.0		21.0	33.8
	5						8.15	5.2		19.7	32.0	8.11	5.5		20.2	33.1	8.11	4.5		21.5	34.0	8.10	4.9		21.0	34.1
0.05	1	8.08	5.4	0.13	18.0	32.0	8.14	5.2	0.12	19.8	32.0	8.13	5.4	0.14	20.1	33.6	8.13	4.5	0.13	21.7	34.8	8.12	5.0	0.13	20.9	34.1
	2						8.15	5.2		19.8	32.0	8.14	5.6		20.2	32.7	8.15	4.4		21.6	33.6	8.13	5.0		21.1	34.1
	3						8.13	5.2		19.6	32.0	8.11	5.6		20.2	32.8	8.13	4.5		21.6	33.6	8.14	5.1		21.1	34.3
	4						8.10	5.0		19.6	32.0	8.11	5.6		20.1	32.3	8.12	4.5		21.4	32.8	8.12	5.1		20.0	34.2
	5						8.04	5.1		19.5	32.0	8.08	5.5		20.1	32.4	8.06	4.5		21.3	33.3	8.10	5.0		20.0	34.0
0.1	1	8.06	5.4	0.25	18.0	32.0	8.02	5.0	0.19	19.6	32.0	8.09	5.4	0.29	20.2	33.1	8.06	4.6	0.23	21.7	33.9	8.12	5.0	0.24	21.0	35.1
	2						7.92	5.0		19.6	32.0	8.03	5.4		20.1	33.1	8.02	4.4		21.5	34.1	8.10	5.1		21.0	35.0
	3						7.99	4.9		19.5	32.0	8.10	5.3		19.9	33.0	8.13	4.4		21.3	35.0	8.13	4.9		20.9	35.1
	4						8.00	5.0		19.4	32.0	8.10	5.3		19.9	33.3	8.10	4.5		21.2	34.7	8.10	5.0		20.9	35.1
	5						8.02	5.0		19.3	32.0	8.10	5.3		19.9	33.5	8.16	4.6		21.1	35.4	8.09	5.0		20.9	35.7
0.2	1	8.04	5.2	0.61	18.0	32.0	7.91	5.0	0.38	19.6	32.0	8.11	5.4	0.38	20.0	32.6	8.14	4.8	0.41	21.5	34.2	8.18	4.9	0.52	21.0	34.8
	2						7.75	4.4		19.1	32.0	8.07	5.4		19.6	36.0	8.05	4.6		20.9	41.1	8.21	5.0		21.0	41.2
	3						7.58	3.8		19.0	32.0	8.04	5.5		19.5	35.2	8.04	4.5		20.7	38.7	8.20	5.0		21.1	38.7
	4						7.76	4.2		18.9	32.0	8.06	5.5		19.6	35.6	8.05	4.5		20.9	38.3	8.17	5.1		21.0	38.9
	5						7.81	4.4		19.0	32.0	8.07	5.4		19.5	35.0	8.11	4.5		20.9	35.9	8.17	5.1		21.0	36.2
0.4	1	8.02	5.2	1.17	18.0	32.0	7.83	4.2	0.71	19.5	32.0	8.16	5.4	0.74	19.9	32.9	8.20	4.6	0.82	21.4	34.0	8.21	5.1	1.09	20.9	34.8
	2						7.87	4.6		19.5	32.0	8.18	5.4		19.9	32.9	8.20	4.6		21.0	33.7	8.18	5.2		20.9	34.0
	3						7.73	3.8		19.5	32.0	8.19	5.2		19.9	33.0	8.20	4.6		21.2	33.8	8.19	5.1		20.9	33.9
	4						7.79	4.8		19.4	32.0	8.17	5.1		19.9	32.9	8.15	4.5		21.2	33.5	8.21	5.1		20.8	33.9
	5						7.91	4.4		19.4	32.0	8.19	5.1		19.9	33.0	8.20	4.5		21.0	33.6	8.21	5.1		20.8	33.9
0.8	1	7.92	5.3	3.62	19.9	32.0	7.62	3.8	1.52	19.5	32.0	8.22	5.3	1.38	19.9	33.2	8.23	4.6	1.42	21.3	33.9	8.22	5.1	1.53	21.0	34.1
	2						7.70	3.4		19.5	32.0	8.21	5.2		19.9	32.4	8.21	4.5		21.2	33.5	8.22	5.0		21.1	34.2
	3						7.61	3.4		19.4	32.0	8.19	5.1		19.9	33.2	8.19	4.4		21.1	34.0	8.21	5.0		21.0	34.7
	4						7.82	3.8		19.4	32.0	8.22	5.0		19.9	32.9	8.23	4.4		21.2	34.0	8.27	5.1		21.0	34.7
	5						7.59	3.0		19.4	32.0	8.24	5.0		19.9	33.0	8.23	4.4		21.2	34.0	8.24	5.0		21.0	34.2
1.6	1	7.88	5.2	7.14	20.2	32.0	7.61	1.4	3.27	19.6	32.0	8.25	5.2	3.45	20.1	32.7	8.23	4.6	3.27	21.3	33.8	8.28	4.9	3.12	21.1	34.1
	2						7.67	1.8		19.4	32.0	8.25	5.1		19.9	32.9	8.22	4.5		21.1	33.7	8.24	4.9		21.1	34.2
	3						7.68	1.8		18.6	32.0	8.15	5.0		19.5	34.4	—	—	—	—	—	—	—	—	—	—
	4						7.51	0.4		19.1	32.0	8.24	5.0		19.6	32.4	—	—	—	—	—	—	—	—	—	—
	5						7.70	2.4		18.9	32.0	8.19	5.0		19.4	36.1	8.12	4.5		20.6	40.8	8.31	5.0		20.9	33.9
Min		7.88	5.2	0.13	18.0	32.0	7.51	0.4	<0.01	18.6	32.0	8.03	5.0	0.14	19.4	32.3	8.02	4.4	<0.10	20.6	32.8	8.07	4.9	<0.10	20.0	33.8
Max		8.08	5.4	7.14	20.2	32.0	8.16	5.2	3.27	19.9	32.0	8.25	5.6	3.45	20.2	36.1	8.23	4.8	3.27	21.7	41.1	8.31	5.2	3.12	21.1	41.2

Note: — = All animals dead.

APPENDIX TABLE 6 (Cont'd)

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration		Day 0					Day 1					Day 2					Day 3					Day 4				
(%)	Rep	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.06	5.4		18.0	32.0	8.14	5.2	<0.01	19.6	32.0	8.11	5.1		19.8	33.0	8.11	4.6	<0.10	21.7	33.9	8.08	4.9	<0.10	21.1	34.1
	2						8.13	5.2		19.9	32.0	8.08	5.2		20.1	33.0	8.07	4.6		21.6	33.6	8.07	5.1		21.1	34.1
	3						8.16	5.1		19.7	32.0	8.12	5.4		20.2	33.6	8.11	4.5		21.6	34.7	8.09	5.1		21.1	34.0
	4						8.16	5.2		19.7	32.0	8.12	5.4		20.2	33.3	8.14	4.5		21.6	33.9	8.12	5.0		21.0	33.8
	5						8.15	5.2		19.7	32.0	8.11	5.5		20.2	33.1	8.11	4.5		21.5	34.0	8.10	4.9		21.0	34.1
0.05	1	8.04	5.2	0.13	19.9	32.0	8.00	5.0	0.11	19.2	32.0	8.11	4.9	0.12	19.9	32.7	8.12	4.6	0.12	21.1	33.6	8.18	5.0	0.11	21.0	34.1
	2						7.97	4.8		19.1	32.0	8.09	4.9		19.6	33.0	8.08	4.5		20.9	33.7	8.19	5.1		21.1	34.2
	3						7.96	4.8		18.9	32.0	8.07	4.8		19.4	34.0	8.06	4.4		20.6	34.7	8.22	5.1		21.1	34.1
	4						7.96	4.8		18.6	32.0	8.08	4.8		19.2	34.2	8.05	4.4		20.4	35.8	8.21	5.1		21.1	34.1
	5						8.03	4.9		18.6	32.0	8.09	4.8		19.3	34.4	8.04	4.5		20.4	36.6	8.19	5.0		21.0	34.2
0.1	1	8.05	5.2	0.25	19.6	32.0	8.00	5.0	0.18	19.1	32.0	8.12	4.9	0.16	19.6	34.7	8.15	4.4	0.17	20.9	36.0	8.19	5.0	0.17	21.0	36.3
	2						7.97	5.0		19.1	32.0	8.15	5.0		19.6	34.6	8.15	4.5		20.7	33.7	8.20	5.0		21.1	36.4
	3						8.01	5.0		18.9	32.0	8.15	4.9		19.4	35.4	8.15	4.6		20.5	34.7	8.16	5.0		21.1	34.7
	4						7.97	4.9		18.8	32.0	8.15	4.9		19.4	35.2	8.14	4.4		20.3	36.0	8.17	5.0		21.1	35.2
	5						8.07	4.9		18.7	32.0	8.17	5.0		19.3	34.6	8.18	4.4		20.2	39.0	8.19	5.0		21.1	39.7
0.2	1	7.96	5.2	0.61	20.1	32.0	7.74	4.4	0.57	19.0	32.0	8.16	5.0	0.30	18.7	31.8	8.14	4.4	0.32	19.4	32.5	8.21	5.0	0.39	21.1	33.4
	2						7.78	4.6		19.1	32.0	8.15	4.9		18.7	32.5	8.13	4.5		19.4	32.8	8.09	5.1		21.0	34.2
	3						7.81	4.5		18.9	32.0	8.14	5.0		18.6	32.4	8.15	4.4		19.2	32.9	8.21	4.9		21.0	34.1
	4						7.85	4.6		18.8	32.0	8.16	5.0		18.4	32.4	8.16	4.4		19.1	33.7	8.23	4.9		21.0	34.1
	5						7.81	4.6		18.6	32.0	8.15	5.0		18.4	33.7	8.15	4.5		19.1	35.1	8.16	5.1		21.1	34.2
0.4	1	7.92	5.2	1.17	20.2	32.0	7.76	3.6	1.08	19.1	31.0	8.15	5.0	1.10	18.9	31.5	8.19	4.6	1.20	19.5	32.4	8.23	5.1	1.16	21.1	33.7
	2						7.75	3.6		19.1	32.0	8.16	5.0		18.6	33.9	8.14	4.5		19.5	35.9	8.18	5.1		21.1	36.2
	3						7.59	1.8		18.7	32.0	8.14	5.0		18.4	34.1	8.10	4.4		19.2	36.5	8.19	5.1		21.1	37.0
	4						7.73	3.4		18.6	32.0	8.16	5.0		18.4	33.7	8.14	4.3		19.2	35.1	8.19	5.0		21.1	36.1
	5						7.80	3.6		18.6	32.0	8.16	5.0		18.5	33.8	8.16	4.3		19.2	35.6	8.22	5.0		21.1	36.1
0.8	1	7.79	5.2	3.62	20.2	32.0	7.52	1.2	2.17	19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.61	1.8		19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.54	2.2		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.71	2.2		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.66	2.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.6	1	7.67	5.0	7.14	20.0	32.0	7.58	2.8	4.43	19.0	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.39	2.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.46	1.4		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.38	1.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.49	1.6		18.9	32.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		7.67	5.0	0.13	18.0	32.0	7.38	1.2	<0.01	18.6	31.0	8.07	4.8	0.12	18.4	31.5	8.04	4.3	<0.10	19.1	32.4	8.07	4.9	<0.10	21.0	33.4
Max		8.06	5.4	7.14	20.2	32.0	8.16	5.2	4.43	19.9	32.0	8.17	5.5	1.10	20.2	35.4	8.19	4.6	1.20	21.7	39.0	8.23	5.1	1.16	21.1	39.7

Note: — = All animals dead.

APPENDIX TABLE 7

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	10	100	
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	9	90	92.0
0.05	1	10	9	9	9	9	90	
	2	10	10	10	9	8	80	
	3	10	10	8	8	7	70	
	4	10	9	7	7	6	60	
	5	10	10	9	8	9	90	78.0
0.1	1	10	6	5	2	6	60	
	2	10	10	9	5	8	80	
	3	10	8	8	7	6	60	
	4	10	8	6	7	8	80	
	5	10	9	8	8	6	60	68.0
0.2	1	10	9	8	4	7	70	
	2	10	8	7	5	7	70	
	3	10	9	7	7	8	80	
	4	10	9	8	7	8	80	
	5	10	10	9	8	8	80	76.0
0.4	1	10	8	7	5	6	60	
	2	10	8	7	6	6	60	
	3	10	8	8	6	6	60	
	4	10	8	7	7	8	80	
	5	10	10	9	8	7	70	66.0
0.8	1	10	5	*	*	3	30	
	2	10	4	*	*	3	30	
	3	10	6	*	*	3	30	
	4	10	4	*	*	3	30	
	5	10	3	*	*	0	0	24.0
1.6	1	10	3	*	*	0	0	
	2	10	2	*	*	0	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	1	*	*	0	0	0.0

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 7 (Cont'd)

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	10	100	
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	10	10	9	90	92.0
0.05	1	10	10	10	10	9	90	
	2	10	9	9	8	6	60	
	3	10	10	9	8	7	70	
	4	10	8	8	8	5	50	
	5	10	9	8	8	6	60	66.0
0.1	1	10	7	7	7	6	60	
	2	10	8	7	5	4	40	
	3	10	7	6	4	7	70	
	4	10	8	7	4	4	40	
	5	10	7	7	6	3	30	48.0
0.2	1	10	6	4	2	2	20	
	2	10	5	5	4	2	20	
	3	10	6	6	3	5	50	
	4	10	6	6	4	6	60	
	5	10	5	4	2	4	40	38.0
0.4	1	10	5	*	*	1	10	
	2	10	3	*	*	2	20	
	3	10	4	*	*	1	10	
	4	10	3	*	*	0	0	
	5	10	3	*	*	0	0	8.0
0.8	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0
1.6	1	10	0	—	—	—	0	
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	0.0

Notes: — = All animals dead.
* Sample too turbid to do counts.

APPENDIX TABLE 8

Mysidopsis bahia
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration		Day 0				Day 1				Day 2				Day 3				Day 4			
(mg/L)	Rep	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal
Control	**1**	8.03	5.6	20.9	32.0	8.00	4.8	21.2	32.0	7.67	5.4	21.6	33.0	7.90	3.8	21.6	33.9	7.93	4.1	21.1	34.0
	2					8.02	4.8	21.2	32.0	7.72	5.4	21.5	33.0	7.91	3.7	21.6	30.9	7.94	4.0	21.1	34.1
	3					8.03	4.8	21.3	32.0	7.70	5.3	21.6	33.0	7.90	3.8	21.8	33.8	7.94	4.0	21.1	34.2
1.25	**1**	8.04	5.4	20.9	32.0	8.00	4.8	21.3	32.0	7.58	5.2	21.6	33.0	7.90	3.6	21.8	33.8	7.94	4.0	20.9	34.1
	2					8.02	4.8	21.2	32.0	7.54	5.1	21.6	33.0	7.93	3.5	21.8	33.7	7.93	4.0	21.0	34.3
	3					8.03	4.8	21.2	32.0	7.38	5.1	21.6	33.0	7.95	3.5	21.7	33.8	7.95	3.9	21.0	34.7
2.5	**1**	8.04	5.4	20.9	32.0	8.01	4.8	21.3	32.0	7.62	5.1	21.6	33.0	7.96	3.6	21.8	33.8	7.99	3.9	20.9	34.1
	2					8.02	4.8	21.1	32.0	7.42	5.1	21.6	33.0	7.93	3.6	21.8	33.6	7.92	3.8	20.9	34.0
	3					8.02	4.6	21.1	32.0	7.47	5.0	21.6	33.0	7.93	3.6	21.7	33.9	7.91	3.8	21.0	33.9
5	**1**	8.04	5.4	21.1	32.0	8.00	4.8	21.1	32.0	7.32	4.7	21.6	33.0	7.98	3.7	21.8	33.1	7.92	3.8	21.0	33.8
	2					8.00	4.7	21.1	32.0	7.38	4.8	21.6	33.0	7.92	3.5	21.8	33.0	7.92	3.9	21.0	33.7
	3					7.98	4.7	21.1	32.0	7.31	4.6	21.5	33.0	7.92	3.5	21.8	33.9	7.91	3.9	21.0	33.9
10	**1**	8.03	5.4	21.2	32.0	7.91	4.6	21.2	32.0	7.30	4.1	21.5	33.0	7.86	3.6	21.9	33.7	7.89	3.9	20.9	34.0
	2					7.91	4.5	21.2	32.0	7.31	4.2	21.5	33.0	7.88	3.6	21.9	33.8	7.89	3.9	20.9	33.9
	3					7.91	4.3	21.2	32.0	7.31	4.2	21.6	33.0	7.87	3.6	22.0	33.6	7.91	3.9	21.0	34.1
20	**1**	8.02	5.3	20.8	32.0	7.85	4.4	20.9	32.0	7.20	4.0	21.6	33.0	7.78	3.7	21.8	33.4	7.90	3.9	21.0	33.9
	2					7.85	4.4	20.9	32.0	7.21	4.0	21.6	33.0	7.75	3.8	21.8	33.4	7.88	3.8	21.0	33.4
	3					7.86	4.2	20.9	32.0	7.21	4.0	21.5	33.0	7.78	3.8	21.8	33.2	7.88	3.9	21.0	33.9
Min		8.02	5.3	20.8	32.0	7.85	4.2	20.9	32.0	7.20	4.0	21.5	33.0	7.75	3.5	21.6	30.9	7.88	3.8	20.9	33.4
Max		8.04	5.6	21.2	32.0	8.03	4.8	21.3	32.0	7.72	5.4	21.6	33.0	7.98	3.8	22.0	33.9	7.99	4.1	21.1	34.7

APPENDIX TABLE 9

Mysidopsis bahia
SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	9	9	90	
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	90.0
1.25	1	10	9	9	8	7	70	
	2	10	10	9	6	6	60	
	3	10	9	8	8	8	80	70.0
2.5	1	10	9	8	6	5	50	
	2	10	10	8	6	6	60	
	3	10	10	8	6	6	60	56.7
5	1	10	11	9	5	5	50	
	2	10	9	7	5	4	40	
	3	10	10	9	7	5	50	46.7
10	1	10	10	9	7	5	50	
	2	10	9	9	4	4	40	
	3	10	9	7	5	5	50	46.7
20	1	10	7	5	3	2	20	
	2	10	10	8	7	5	50	
	3	10	10	8	5	4	40	36.7

APPENDIX TABLE 10

Strongylocentrotus purpuratus
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/11/94

Site	Concentration (%)	Day 0 °C	Day 0 DO	Day 0 pH	Day 0 Sal	Day 1 °C	Day 1 DO	Day 1 pH	Day 1 Sal	Day 2 °C	Day 2 DO	Day 2 pH	Day 2 Sal	Day 3 °C	Day 3 DO	Day 3 pH	Day 3 Sal	Day 4 °C	Day 4 DO	Day 4 pH	Day 4 Sal
Control		16.3	8.0	7.49	26	15.1	8.7	7.77	27	16.2	8.4	7.87	26	15.4	8.4	7.79	26	15.7	8.2	7.89	27
HSW-1	0.08	16.0	8.1	7.42	26	14.5	8.6	7.62	27	15.6	8.4	7.86	26	15.6	7.7	7.84	26	15.9	8.1	7.88	26
	0.15	16.0	8.0	7.43	27	14.5	6.6	7.51	27	15.5	7.4	7.80	27	15.6	6.5	7.80	27	15.7	8.1	7.85	27
	0.3	16.2	8.0	7.83	29	14.5	4.5	7.54	29	15.7	2.2	7.59	28	15.5	3.0	7.47	28	15.8	7.8	7.65	29
	0.6	16.2	8.0	7.51	26	14.5	4.1	7.51	27	15.9	2.3	7.56	26	15.6	2.7	7.49	26	15.7	7.4	7.93	27
	1.2	16.4	8.0	7.62	26	14.5	1.5	7.10	29	15.6	1.3	7.46	28	15.7	1.7	7.51	27	15.1	7.4	7.97	29
HSW-2	0.08	16.2	8.0	7.33	26	14.5	1.2	7.41	27	15.3	7.7	7.93	27	15.6	7.9	7.80	27	15.2	7.6	7.95	27
	0.15	16.4	8.0	7.34	27	14.5	1.6	7.42	27	15.5	7.7	7.96	27	15.7	7.3	7.77	27	15.0	7.8	7.95	27
	0.3	16.4	8.0	7.21	27	14.5	1.3	7.45	27	15.6	7.8	7.82	27	15.6	6.9	7.79	27	15.0	7.8	7.97	27
	0.6	16.0	8.0	7.21	26	15.7	1.3	7.42	27	16.2	3.0	7.52	27	15.7	2.7	7.47	27	16.2	6.6	7.71	27
	1.2	16.2	7.9	6.87	26	15.7	1.3	7.10	27	16.1	1.4	7.42	27	15.7	1.7	7.38	27	16.2	6.4	7.63	27
	Min	16.0	7.9	6.87	26	14.5	1.2	7.10	27	15.3	1.3	7.42	26	15.4	1.7	7.38	26	15.0	6.4	7.63	26
	Max	16.4	8.1	7.83	29	15.7	8.7	7.77	29	16.2	8.4	7.96	28	15.7	8.4	7.84	28	16.2	8.2	7.97	29

APPENDIX TABLE 11

Strongylocentrotus purpuratus

SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE EFFLUENT TEST

Test Dates: 4/7-4/11/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	156		31.2			
	2	136		27.2			
	3	141		28.2			
	4	168		33.6			
	5	137		27.4			
	Mean			29.5			
Final Control	1	95	14	21.8		12.8	
	2	59	4	12.6		6.3	
	3	109	7	23.2		6.0	
	4	94	1	19.0		1.1	
	5	90	2	18.4		2.2	
	Mean			19.0	64.4	5.7	NA
HSW-1							
0.08	1	45	32	15.4		41.6	
	2	63	53	23.2		45.7	
	3	66	43	21.8		39.4	
	4	76	38	22.8		33.3	
	5	78	40	23.6		33.9	
	Mean			21.4	72.4	38.8	0.0
0.15	1	0	79	15.8		100.0	
	2	0	48	9.6		100.0	
	3	0	44	8.8		100.0	
	4	0	89	17.8		100.0	
	5	0	99	19.8		100.0	
	Mean			14.4	48.7	100.0	24.4
0.3	1	0	50	10.0		100.0	
	2	0	53	10.6		100.0	
	3	0	57	11.4		100.0	
	4	0	84	16.8		100.0	
	5	0	58	11.6		100.0	
	Mean			12.1	40.9	100.0	36.4
0.6	1	0	66	13.2		100.0	
	2	0	85	17.0		100.0	
	3	0	74	14.8		100.0	
	4	0	112	22.4		100.0	
	5	0	57	11.4		100.0	
	Mean			15.8	53.4	100.0	17.1
1.2	1	0	106	21.2		100.0	
	2	0	115	23.0		100.0	
	3	0	92	18.4		100.0	
	4	0	60	12.0		100.0	
	5	0	114	22.8		100.0	
	Mean			19.5	66.0	100.0	100.0

APPENDIX TABLE 11 (Cont'd)

Strongylocentrotus purpuratus
SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE
EFFLUENT TEST
Test Dates: 4/7-4/11/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2							
0.08	1	0	63	12.6		100.0	
	2	0	61	12.2		100.0	
	3	0	39	7.8		100.0	
	4	0	36	7.2		100.0	
	5	0	58	11.6		100.0	
	Mean			10.3	34.8	100.0	45.9
0.15	1	0	101	20.2		100.0	
	2	0	112	22.4		100.0	
	3	0	129	25.8		100.0	
	4	0	122	24.4		100.0	
	5	0	130	26.0		100.0	
	Mean			23.8	80.5	100.0	0.0
0.3	1	0	89	17.8		100.0	
	2	0	128	25.6		100.0	
	3	0	119	23.8		100.0	
	4	0	119	23.8		100.0	
	5	0	91	18.2		100.0	
	Mean			21.8	74.0	100.0	0.0
0.6	1	0	116	23.2		100.0	
	2	0	119	23.8		100.0	
	3	0	113	22.6		100.0	
	4	0	79	15.8		100.0	
	5	0	104	20.8		100.0	
	Mean			21.2	72.0	100.0	0.0
1.2	1	0	76	15.2		100.0	
	2	0	87	17.4		100.0	
	3	0	92	18.4		100.0	
	4	0	88	17.6		100.0	
	5	0	76	15.2		100.0	
	Mean			16.8	56.8	100.0	11.8

APPENDIX TABLE 12

Strongylocentrotus purpuratus
WATER QUALITY MEASUREMENTS FOR THE REFERENCE TOXICANT (C0PPER) TEST
Test Dates: 4/7-4/11/94

Concentration	Day 0				Day 1				Day 2				Day 3				Day 4			
(µg/L)	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal	°C	DO	pH	Sal
0.1	15.6	8.9	7.88	29	14.3	NT	NT	NT	14.2	8.1	7.97	29	14.4	8.4	8.01	29	15.0	7.6	7.98	29
0.32	15.8	8.9	7.90	29	14.3	NT	NT	NT	14.2	8.1	8.00	29	14.4	8.4	8.04	29	15.0	7.7	7.99	29
1.8	15.8	8.9	7.92	29	14.4	NT	NT	NT	14.3	8.3	8.02	29	14.5	8.3	8.06	29	14.9	7.9	8.00	29
18	15.8	9.1	7.80	28	14.3	NT	NT	NT	14.2	8.3	8.01	28	14.5	8.3	8.06	29	15.0	7.9	8.00	29
56	15.8	9.1	7.86	26	14.4	NT	NT	NT	14.2	8.6	8.02	25	14.5	8.3	8.06	29	15.0	8.0	8.01	25
Min	15.6	8.9	7.80	26	14.3				14.2	8.1	7.97	25	14.4	8.3	8.01	29	14.9	7.6	7.98	25
Max	15.8	9.1	7.92	29	14.4				14.3	8.6	8.02	29	14.5	8.4	8.06	29	15.0	8.0	8.01	29

Note: NT = Not taken.

APPENDIX TABLE 13

Strongylocentrotus purpuratus
SUMMARY OF SURVIVAL AND DEVELOPMENT FOR THE ECHINODERM LARVAE
REFERENCE TOXICANT (Copper) TEST
Test Dates: 4/7-4/11/94

Concentration (µg/L)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Copper							
0.1	1	78	14	18.4		15.2	
	2	86	19	21.0		18.1	
	3	86	12	19.6		12.2	
	Mean			19.7	66.7	15.2	0.0
0.32	1	26	1	5.4		3.7	
	2	33	1	6.8		2.9	
	3	96	0	19.2		0.0	
	Mean			10.5	35.5	2.2	44.9
1.8	1	69	4	14.6		5.5	
	2	60	2	12.4		3.2	
	3	96	4	20.0		4.0	
	Mean			15.7	53.1	4.2	17.5
18	1	3	51	10.8		94.4	
	2	0	31	6.2		100.0	
	3	0	28	5.6		100.0	
	Mean			7.5	25.5	98.1	60.4
56	1	0	38	7.6		100.0	
	2	0	24	4.8		100.0	
	3	0	48	9.6		100.0	
	Mean			7.3	24.9	100.0	61.4

APPENDIX TABLE 14

Mytilus edulis
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Day 0 °C	Day 0 DO	Day 0 pH	Day 0 Sal	Day 1 °C	Day 2 °C	Day 2 DO	Day 2 pH	Day 2 Sal
Control	1	16.3	8.0	7.49	26	14.8	16.0	7.2	7.79	26
	2					14.6	16.0	7.2	7.82	26
	3					14.5	16.0	7.5	7.82	26
	4					14.7	16.0	7.5	7.88	26
	5					14.8	16.0	7.6	7.96	26
HSW-1										
0.08	1	16.0	8.1	7.42	26	14.5	16.0	7.6	7.68	26
	2					14.5	16.0	7.5	7.65	26
	3					14.4	16.1	7.3	7.67	26
	4					14.5	16.0	7.2	7.66	26
	5					14.5	16.1	7.1	7.66	26
0.15	1	16.0	8.0	7.43	27	14.5	16.0	4.0	7.46	26
	2					14.4	16.0	4.0	7.40	26
	3					14.4	16.0	3.8	7.38	26
	4					14.4	16.0	3.8	7.38	26
	5					14.5	16.0	3.6	7.40	26
0.3	1	16.2	8.0	7.83	29	14.4	16.0	2.0	7.44	28
	2					14.5	16.0	2.0	7.52	28
	3					14.5	16.0	1.8	7.54	28
	4					14.4	16.0	1.8	7.56	28
	5					14.5	16.0	1.5	7.55	28
0.6	1	16.2	8.0	7.51	26	14.5	16.0	1.6	7.56	26
	2					14.5	16.0	1.7	7.58	26
	3					14.5	16.0	1.7	7.60	26
	4					14.6	16.1	2.1	7.61	26
	5					14.5	16.1	2.0	7.60	26
1.2	1	16.4	8.0	7.62	26	14.4	16.0	4.2	7.62	26
	2					14.5	16.0	4.4	7.67	26
	3					14.5	16.0	4.3	7.64	26
	4					14.5	16.1	4.5	7.67	26
	5					14.5	16.1	4.6	7.83	26
	Min	16.0	8.0	7.42	26	14.4	16.0	1.5	7.38	26
	Max	16.4	8.1	7.83	29	14.8	16.1	7.6	7.96	28

APPENDIX TABLE 14 (Cont'd)

Mytilus edulis
WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST
Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1	Day 2			
(%)	Rep	°C	DO	pH	Sal	°C	°C	DO	pH	Sal
HSW-2										
0.08	1	16.2	8.0	7.33	26	14.5	16.0	7.4	7.93	26
	2					14.6	16.0	7.7	7.92	26
	3					14.5	16.0	7.5	7.95	26
	4					14.5	16.1	7.5	7.97	26
	5					14.5	16.1	7.6	7.98	27
0.15	1	16.4	8.0	7.34	27	14.5	16.0	7.8	7.91	26
	2					14.5	16.0	8.0	7.94	26
	3					14.4	16.1	8.0	7.94	26
	4					14.5	16.1	7.9	7.86	26
	5					14.5	16.1	7.7	7.85	26
0.3	1	16.4	8.0	7.21	27	14.5	16.0	7.7	7.83	26
	2					14.5	16.0	7.7	7.86	26
	3					14.5	16.0	7.7	7.77	26
	4					14.5	16.1	7.6	7.59	26
	5					14.5	16.1	7.2	7.62	26
0.6	1	16.0	8.0	7.21	26	14.5	16.0	1.7	7.56	26
	2					14.6	16.1	1.7	7.53	26
	3					14.5	16.1	1.8	7.51	26
	4					14.6	16.1	1.8	7.51	26
	5					14.5	16.1	1.8	7.50	26
1.2	1	16.2	7.9	6.87	26	14.5	16.0	2.0	7.47	26
	2					14.5	16.1	1.7	7.37	26
	3					14.5	16.1	1.6	7.39	26
	4					14.5	16.1	2.0	7.42	26
	5					14.5	16.1	2.0	7.45	26
	Min	16.0	7.9	6.87	26	14.4	16.0	1.6	7.37	26
	Max	16.4	8.0	7.34	27	14.6	16.1	8.0	7.98	27

APPENDIX TABLE 15

Mytilus edulis
SUMMARY OF RESULTS FOR BIVALVE LARVAE BIOASSAY
Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	129		25.8			
	2	95		19.0			
	3	102		20.4			
	4	76		15.2			
	5	115		23.0			
	Mean			20.7			
Final Control	1	103	13	23.2		11.2	
	2	97	3	20.0		3.0	
	3	86	5	18.2		5.5	
	4	83	5	17.6		5.7	
	5	106	7	22.6		6.2	
	Mean			20.3	98.2	6.3	NA
HSW-1							
0.08	1	22	61	16.6		73.5	
	2	2	78	16.0		97.5	
	3	0	72	14.4		100.0	
	4	0	77	15.4		100.0	
	5	5	67	14.4		93.1	
	Mean			15.4	74.2	92.8	24.3
0.15	1	0	74	14.8		100.0	
	2	0	76	15.2		100.0	
	3	0	64	12.8		100.0	
	4	0	86	17.2		100.0	
	5	0	61	12.2		100.0	
	Mean			14.4	69.8	100.0	28.9
0.3	1	0	139	27.8		100.0	
	2	0	120	24.0		100.0	
	3	0	133	26.6		100.0	
	4	0	91	18.2		100.0	
	5	0	82	16.4		100.0	
	Mean			22.6	100.0	100.0	0.0
0.6	1	0	73	14.6		100.0	
	2	0	133	26.6		100.0	
	3	0	90	18.0		100.0	
	4	0	96	19.2		100.0	
	5	0	93	18.6		100.0	
	Mean			19.4	93.7	100.0	4.4
1.2	1	0	90	18.0		100.0	
	2	0	75	15.0		100.0	
	3	0	87	17.4		100.0	
	4	0	80	16.0		100.0	
	5	0	91	18.2		100.0	
	Mean			16.9	81.7		16.7

APPENDIX TABLE 15 (Cont'd)

Mytilus edulis
SUMMARY OF RESULTS FOR BIVALVE LARVAE BIOASSAY
Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2							
0.08	1	0	109	21.8		100.0	
	2	1	84	17.0		98.8	
	3	0	100	20.0		100.0	
	4	0	110	22.0		100.0	
	5	0	95	19.0		100.0	
	Mean			20.0	96.4	99.8	1.7
0.15	1	0	100	20.0		100.0	
	2	0	90	18.0		100.0	
	3	0	111	22.2		100.0	
	4	0	89	17.8		100.0	
	5	0	115	23.0		100.0	
	Mean			20.2	97.6	100.0	0.5
0.3	1	0	82	16.4		100.0	
	2	0	101	20.2		100.0	
	3	0	97	19.4		100.0	
	4	0	89	17.8		100.0	
	5	0	104	20.8		100.0	
	Mean			18.9	91.4	100.0	6.8
0.6	1	0	144	28.8		100.0	
	2	0	128	25.6		100.0	
	3	0	94	18.8		100.0	
	4	0	103	20.6		100.0	
	5	0	119	23.8		100.0	
	Mean			23.5	100.0	100.0	0.0
1.2	1	0	81	16.2		100.0	
	2	0	94	18.8		100.0	
	3	0	104	20.8		100.0	
	4	0	88	17.6		100.0	
	5	0	87	17.4		100.0	
	Mean			18.2	87.7	100.0	10.5

APPENDIX TABLE 16

Mytilus edulis
WATER QUALITY MEASUREMENTS
FOR THE REFERENCE TOXICANT (C0PPER) TEST
Test Dates: 4/7-4/9/94

Concentration		Day 0				Day 1	Day 2			
µg/L	Rep	°C	DO	pH	Sal	°C	°C	DO	pH	Sal
0.56	1	15.8	9.2	7.91	30	14.3	14.0	7.7	7.95	28
	2					14.3	14.0	7.8	7.96	29
	3					14.3	14.0	7.9	7.96	29
3.2	1	15.7	8.9	7.91	29	14.3	14.1	7.9	7.96	28
	2					14.3	14.0	7.9	7.96	29
	3					14.2	14.0	8.1	7.96	29
10	1	15.6	8.7	7.92	29	14.3	14.0	8.0	7.96	28
	2					14.4	14.1	8.0	7.97	28
	3					14.3	14.1	8.1	7.97	28
32	1	15.6	9.7	7.78	26	14.3	14.0	8.0	7.97	26
	2					14.3	14.1	8.1	7.96	26
	3					14.3	14.1	8.1	7.95	26
56	1	15.8	9.1	7.86	26	14.4	14.0	8.3	7.95	25
	2					14.3	14.0	8.1	7.96	25
	3					14.4	14.0	8.1	7.96	25
	Min	15.6	8.7	7.78	26	14.2	14.0	7.7	7.95	25
	Max	15.8	9.7	7.92	30	14.4	14.1	8.3	7.97	29

APPENDIX TABLE 17

Mytilus edulis
SUMMARY OF RESULTS FOR THE BIVALVE LARVAE
REFERENCE TOXICANT (COPPER) BIOASSAY
Test Dates: 4/7-4/9/94

Concentration (µg/L)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
0.56	1	92	5	19.4		5.2	
	2	76	3	15.8		3.8	
	3	86	6	18.4		6.5	
	Mean			17.9	86.3	5.2	12.0
3.2	1	99	24	24.6		19.5	
	2	95	22	23.4		18.8	
	3	89	17	21.2		16.0	
	Mean			23.1	100.0	18.1	0.0
10	1	88	16	20.8		15.4	
	2	11	91	20.4		89.2	
	3	29	45	14.8		60.8	
	Mean			18.7	90.2	55.1	8.0
32	1	0	34	6.8		100.0	
	2	0	12	2.4		100.0	
	3	0	50	10.0		100.0	
	Mean			6.4	30.9	100.0	68.5
56	1	0	0	0.0		100.0	
	2	0	6	1.2		100.0	
	3	0	13	2.6		100.0	
	Mean			1.3	6.1	100.0	93.8

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